Integrated Masters in Bioengineering

Siloxanes in cosmetics and personal care products

Master's Thesis

of

Daniela Sofia Borges Capela

Developed within the discipline of Dissertation

Conducted at

Laboratory for Process Engineering, Environment, Biotechnology and Energy
Faculty of Engineering, University of Porto

FEUP advisor: **Doctor Vera Homem**

FEUP co-advisor: **Doctor Lúcia Santos**

U.PORTO
FEUP FACULDADE DE ENGENHARIA
UNIVERSIDADE DO PORTO

Department of Chemical Engineering

January, 2016

"Some people succeed because they are destined to, but most people succeed because they are determined to."

By Henry Ford

Acknowledgements

First of all, I would like to thank LEPABE (Laboratory for Process Engineering, Environment, Biotechnology and Energy) and the Department of Chemical Engineering, for providing the conditions, material and equipment to make this work possible.

This work was financially supported by: Project UID/EQU/00511/2013-LEPABE (Laboratory for Process Engineering, Environment, Biotechnology and Energy – EQU/00511) by FEDER funds through Programa Operacional Competitividade e Internacionalização – COMPETE2020 and by national funds through FCT - Fundação para a Ciência e a Tecnologia.

I would like to thank Dr. Vera Homem for believing in me since the beginning, and for always help and supporting me. I also want to thank all the patient she had with me, all the teachings, all the good times provided and especially for being my friend.

I would like to express my gratefulness to my co-advisor, Dr. Lúcia Santos, for the support and availability provided during this thesis.

I also would like to thank the group from the laboratory E201 for all the good moments and animation which allowed a great environment to work, and an especial thanks to José Avelino for the help.

As this acknowledgments cannot be only restrict to the period that I made my thesis but also to the five years that allowed me to get here, I want to thank all the friends that I made during my stay in UTAD. It was a really pleasure to meet all that great people, it was such an awesome time that we spent together.

I could not let pass this opportunity to thank my friends from my ERASMUS program in Cranfield, specially Demetryus, Alicia, Pieter and Shaz. They were always there for me, not only during my stay in the UK but also when I left through our skype conversations and the trips that we made after that. I know I made friends for life. Also from Cranfield but with Spanish origins, I want to thank Alicia Rodríguez, my supervisor, for all the teachings, patient, support and specially for being a great friend.

As people say, “friends are the family you choose”, my thanks go to Mitó, Mary, Ana Maria (also known as Wow), Cláudia, Filipe, Morgana, Denise and Duarte. We spent really good moments together, and those moments were crucial to make this stage unforgettable and something to remember in the future. These guys were fundamental to have some relaxing time and get some extra energy back, especially during our trips to downtown, drinking hot chocolate, and the movie sessions.

As the last ones are always the first, I want to thank my parents for always be there for me and the help they demonstrated during this important stage in my life. I am really grateful for that.

My sincere thanks go to my sister Catarina for always supporting me and help me in every decision that I needed to make, even with lots of kilometres between us, but through these years we learned how to make the distance a minor problem, and continued help each other in everything.

Resumo

Organosiloxanos são compostos amplamente utilizados como emolientes e humectantes em diversas formulações de produtos de higiene pessoal. Eles são particularmente utilizados nas formulações de cremes e loções, sabonetes, e produtos capilares com o intuito de amaciar, suavizar e hidratar. Na verdade, a vasta utilização destes compostos combinada com a sua natureza lipofílica, torna-os alvos interessantes para investigação futura, em particular na área da toxicologia.

O objetivo fulcral deste estudo residiu na determinação dos níveis de concentração dos siloxanos metílicos voláteis (VMSs) nos produtos referentes às marcas mais vendidas na região do Porto (Portugal), permitindo estimar a exposição dérmica a estes siloxanos e quantificar as quantidades libertadas *"down-the-drain"* para o ambiente. Foram investigados oito VMSs, quatro lineares (L2-L5) e quatro cíclicos (D3-D6). Para realizar este trabalho, uma metodologia de QuEChERS ("Quick, Easy, Cheap, Effective, Rugged, and Safe"), que nunca fora previamente testada para a análise de siloxanos em produtos de higiene, foi implementada como um protocolo de preparação de amostras que conjuga metodologia de extração com uma etapa de *"clean-up"*. Esta metodologia envolveu a extração de 500 mg de amostra utilizando hexano (3 mL), seguida por adição de sulfato de magnésio anidro (800 mg) e acetato de sódio (750 mg). A etapa de *"clean-up"* foi efetuada utilizando um polímero contendo uma amina primária e secundária (60 mg) e octadecil-sílica (30 mg). Os extratos obtidos foram analisados por cromatografia gasosa de espectrometria de massa (GC-MS). Os limites de deteção variaram entre 0,17 (L2) e 3,75 $ng.g^{-1}$ (L5) e foram bastante inferiores àqueles descritos na literatura para a deteção de siloxanos em cosméticos e produtos de higiene pessoal. O método demonstrou ser preciso (%RSD < 10%) e exato (recuperação média de 84%).

Cento e trinta e seis produtos de higiene pessoal (formulações para adultos e bebés/crianças) foram analisados e os VMSs foram detetados em 96% das amostras, em concentrações que variaram entre 0,003 $\mu g.g^{-1}$ e 1203,28 $\mu g.g^{-1}$. Os siloxanos cíclicos foram mais frequentemente detetados (94% das amostras) e em concentrações mais elevadas que os lineares. O champô para adultos apresentou a concentração mais elevada para os siloxanos cíclicos (1203,28 $\mu g.g^{-1}$ para o D3), enquanto para o champô de hotel apresentou um maior nível de siloxanos lineares (8,61 $\mu g.g^{-1}$ para L3). Concentrações médias totais mais elevadas foram determinadas em cremes faciais (150,68 $\mu g.g^{-1}$), hidratantes corporais (84,30 $\mu g.g^{-1}$) e champôs para adultos (58,90 $\mu g.g^{-1}$). As loções de bebé e os produtos dentífricos apresentaram os níveis de concentração mais baixos.

Combinando estes resultados com as quantidades usadas diariamente destes produtos, foi estimada uma exposição dérmica média diária de 25,04 $\mu g.kg_{bw}^{-1}.dia^{-1}$ para adultos e 0,35 $\mu g.kg_{bw}^{-1}.dia^{-1}$ para crianças. Os principais responsáveis pela exposição dérmica em adultos foram os hidratantes corporais, cremes faciais e os *"aftershave"*, enquanto para bebés/crianças os principais responsáveis foram os cremes corporais, géis de banho e champôs. Comparando estes níveis com os presentes em estudos de toxicidade dérmica, parece que a exposição cutânea a estes compostos não conduz a riscos de saúde. Foi ainda estimada a quantidade libertada de siloxanos "*down-the-drain*" para os sistemas de esgotos através da utilização destes produtos de higiene. Emissões *per capita* entre 54,71 e 10606,93 $\mu g.dia^{-1}$ (média: 2011,05 $\mu g.dia^{-1}$) foram determinadas e considerando o pior cenário possível, o D5 e D3 foram os siloxanos predominantes nesses efluentes, com emissões máximas de 3690,50 $\mu g.dia^{-1}$ e 3098,25 $\mu g.dia^{-1}$, respetivamente.

Palavras-Chave: Siloxanos metílicos voláteis, produtos de cosmética e higiene pessoal, exposição humana, emissões "*down-the-drain*", QuEChERS, GC-MS.

Abstract

Organosiloxanes are widely employed as emollients and humectants in personal care formulations. They are particularly used in the preparation of creams and lotions, bath soaps and hair care products to soften, smooth, and moisten. Actually, the widespread use of organosiloxanes combined with their lipophilic nature, makes them interesting targets for future research, particularly in the toxicology area.

The main goal of this study was the determination of the concentration levels of volatile methylsiloxanes (VMSs) in the bestselling brands of personal care products (PCPs) in the Oporto region (Portugal), allowing the estimation of human dermal exposure to siloxanes and the quantification of the quantities released "down-the-drain" to the environment. Eight VMSs, four linear (L2-L5) and four cyclic (D3-D6) were investigated. To accomplish this task, a QuEChERS methodology ("Quick, Easy, Cheap, Effective, Rugged, and Safe"), which was never tested for siloxanes in cosmetics, was employed as a sample preparation protocol that couples extraction method with cleanup. This methodology involved the extraction of 500 mg of sample using hexane (3 mL), followed by the addition of anhydrous magnesium sulphate (800 mg) and sodium acetate (750 mg). The clean-up step was performed using primary and secondary amine (60 mg) and octadecyl–silica (30 mg) sorbents. The obtained extracts were analysed by gas chromatography-mass spectrometry (GC-MS). The limits of detection ranged from 0.17 (L2) to 3.75 $ng.g^{-1}$ (L5), being much lower than the values described in the literature for the detection of siloxanes in cosmetics and personal care products. The method showed a high precision (%RSD < 10%) and accuracy (average recovery of 84%).

Hundred and thirty six personal care products (adult and baby/children formulations) were studied and VMSs were detected in 96% of the samples, in concentrations ranging from 0.003 $\mu g.g^{-1}$ to 1,203.28 $\mu g.g^{-1}$. Cyclic siloxanes were more frequently detected (94% of the samples) and at higher concentrations than linear. Adult shampoo exhibited the highest concentration for cyclic siloxanes (1,203.28 $\mu g.g^{-1}$ for D3) and hotel shampoo for linear siloxanes (8.61 $\mu g.g^{-1}$ for L3). Higher mean total concentrations were achieved in facial creams (150.68 $\mu g.g^{-1}$), adult body moisturizers (84.30 $\mu g.g^{-1}$) and adult shampoo (58.90 $\mu g.g^{-1}$). Baby lotions and toothpastes revealed the lowest concentration levels.

Combining these results with the daily usage amounts, an average daily dermal exposure of 25.04 $\mu g.kg_{bw}^{-1}.day^{-1}$ for adults and 0.35 $\mu g.kg_{bw}^{-1}.day^{-1}$ for baby/children was achieved. The main contributors for adults dermal exposure were body moisturizers, followed by facial creams and aftershaves, while for baby/children dermal exposure were body moisturizers, followed by shower gel and shampoo. Comparing these levels with some dermal toxicity studies present in literature, it seems that the dermal exposure to these compounds does not seem to lead to health risks.

An estimation of the amount of siloxanes released “down-the-drain” into the sewage systems through the use of toiletries was also carried out. Emissions *per capita* between 54.71 and 10,606.93 $\mu g.day^{-1}$ (mean: 2,011.05 $\mu g.day^{-1}$) was determined and considering the worst-case scenario, D5 and D3 were the predominant siloxanes in the effluents, with emissions of 3,690.50 $\mu g.day^{-1}$ and 3,098.25 $\mu g.day^{-1}$, respectively.

Keywords: volatile methylsiloxanes, personal care products, human exposure, “down-the-drain” emissions, QuEChERS, GC-MS.

Contents

List of Figures

List of Tables

List of Acronyms

a	slope
A	Amount of Product Applied ($g.event^{-1}$)
Acet	Acetonitrile
b	Intercept
b.p.	Boiling Point (°C)
BW	Body Weight (kg)
C_{18}	Octadecylsilane
C	Siloxane Concentration ($\mu g.g^{-1}$)
CI	Chemical Ionization
cVMS	Cyclic Volatile Methylsiloxane
Cont.	Continuation
D3	Hexamethylcyclotrisiloxane
D4	Octamethylcyclotetrasiloxane
D5	Decamethylcyclotetrasiloxane
D6	Dodecamethylcyclohexasiloxane
DCM	Dichloromethane
D_{exp}	Daily Dermal Exposure ($\mu g.kg_{bw}^{-1}.day^{-1}$)
DVB	Divinylbenzene
dSPE	Dispersive Solid-Phase Extraction
EA	Ethyl acetate
EI	Electron Ionization
E_m	Emission "Down-the-Drain" ($\mu g.day^{-1}$)
F	Frequency of Application ($events.day^{-1}$)
F_{dermal}	Rate of Penetration
F_{evap}	Evaporation Factor
GC	Gas Chromatography
GC-FID	Gas Chromatography-Flame Ionization Detector
GC-MS	Gas Chromatography-Mass Spectrometry
GC-MS/MS	Gas Chromatography-Tandem Mass Spectrometry
Hex	Hexane
HPV	High Production Volume
HS-GC/MS	Headspace-Gas Chromatography-Mass Spectrometry
HS-SPME	Headspace extraction – solid-phase microextraction
i	Type of Toiletry Product
INCI	International Nomenclature for Cosmetics Ingredients
IUPAC	International Union of Pure and Applied Chemistry
j	Type of Siloxane
LC-MS/MS	Liquid Chromatography-Tandem Mass Spectrometry
LLE	Liquid-Liquid Extraction
LLME	Liquid-Liquid Microextraction
LOD	Limit of Detection ($ng.g^{-1}$)
LOQ	Limit of Quantification ($ng.g^{-1}$)
M4Q	Tetrakis Trimethylsiloxysilane-*internal standard*
MASE	Membrane assisted solvent-extraction
MeOH	Methanol
MS	Mass Spectrometry
MSE	Membrane-assisted solvent extraction
n	Number of Personal Care Product
na	Not Available
nd	Not Detected
NOEL	No-Observed-Effect Level ($mg.kg_{bw}^{-1}$)
PCP	Personal Care Products
PDMS	Polydimethylsiloxane
PPCPs	Pharmaceutical and Personal Care Products
PSA	Primary and Secondary Amine
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
R	Correlation Factor
R_i	Retention Factor (dimensionless)
REC	Recovery (%)
RF	Response Factor
RSD	Relative Standard Deviation (%)

RT	Retention Time (min)
Sa	Standard Deviation of the Slope
Sb	Standard Deviation of Intercept
SIS	Selected Ion Storage
S/N	Signal/Noise
SPE	Solid-Phase Extraction
SPME	Solid-Phase Microextraction
STP	Sewage Treatment Plant
U_1	Uncertainty Associated to the Standard Preparation
U_2	Uncertainty Associated to the Calibration Curve
U_3	Uncertainty Associated to the Precision
U_4	Uncertainty Associated to the Accuracy
U_{global}	Global Uncertainty
UNITIS	European Organization of Cosmetics Ingredients Industries and Services
USE	Ultrasound extraction
USA-DLLME	Ultrasound-Assisted Dispersive Liquid-Liquid Microextraction
VMS	Volatile Methylsiloxane
VOC	Volatile Organic Compounds
WWTPs	Wastewater Treatments Plants

1 Background and Presentation of the Project

Throughout history, men and women have utilized cosmetics to increase their beauty, soften skin, protect their health and remove odours. Though used for different purposes, cosmetics have persisted as a historical constant from the Ancient Egyptians to modern-days (Power, 2010). Since the early twentieth century, manufacture of cosmetics has been controlled by a group of a few multi-national corporations. The global cosmetics industry is broken down into six main classes of products such as fragrances, hair care, makeup, oral care, skin care and toiletries (as soaps and shower gels) (Romanowski, 2014), in which skincare is considered the largest one, accounting for 33.8% of the global market in 2012 (Statistics Portal, 2008). In fact, the cosmetic industry seems to be continuously emerging, in particular with the internet companies. Actually, use of cosmetic products is increasing since a good appearance is a portrayal of well-being, success and healthiness.

UNITIS (European organization of cosmetics ingredients industries and services) is an European professional organization that unites companies involved in the field of cosmetics ingredients (e.g. manufacturers, distributors and evaluation companies that test cosmetics and cosmetic ingredients) and whose main objective is master their future nearby regulatory bodies and partners of the cosmetic industry. In accordance with UNITIS, more than 5 billion personal hygiene items are sold every year. Virtually everyone in Europe will use at least one product *per day*, such as soap or toothpaste. In fact, most consumers use several products at the same time, being in contact with the same substance through different sources (Dudzina et al., 2014).

Organosiloxanes are one of the most relevant classes of ingredients incorporated in personal care products, due to their unique properties such as high thermal stability and smooth texture (Horii and Kannan, 2008; Wang et al., 2009). They are extremely used in the formulation of a wide range of cosmetic and personal care products, including creams and lotions, bath soaps, shampoo and hair care products to soften, smooth, and moisten. In fact, they are responsible for the silky and shiny apperance that some personal care products confere to hair and skin. According to International Union of Pure and Applied Chemistry (IUPAC, 2006), organosiloxanes are compounds in which silicon atoms (Si) are linked via oxygen atoms, each silicon bearing one or several organic groups. The US EPA (2007) reported that their annual import and production in the United States of America increased by ten times in the last 25 years to more than 225,000 and 22,500 tonnes, respectively. In Europe, the amounts of organosiloxane octamethylcyclotetrasiloxane (D4), decamethylcyclotetrasiloxane (D5) and dodecamethylcyclohexasiloxane (D6) used in 2004 for personal care applications were estimated in 579, 17,300 and 1,989 tonnes, respectively (UK EA, 2009). This means that D5 represents around 87% of the production for the year 2004.

In fact, the intensive and widespread use of organosiloxanes combined with their lipophilic nature, makes them interesting targets for future research. Therefore, this work focused on determining the concentration levels of these compounds in the bestselling brands of personal care products (PCPs) in the Oporto region (Portugal), allowing the determination of dermal exposure to siloxanes and the evaluation of the “down-the-drain” quantities released into the environment. To accomplish this task, a QuEChERS technique (a portmanteau for "Quick, Easy, Cheap, Effective, Rugged, and Safe") coupled to gas chromatography-mass spectrometry (GC-MS) was employed to analyse siloxanes in personal care products. In fact, this methodology has never been tested for the analyses of this kind of organic compounds in toiletries.

2 Introduction

2.1 Personal care products

Personal care products (PCPs) or toiletries are non-medical consumable products that are proposed to be used in the topical care and grooming of the body and hair and that is rubbed, poured, sprinkled, or sprayed on, introduced into, or otherwise applied to a body, human or animal, for cleansing, beautifying, promoting attractiveness, or altering the appearance without affecting the body's structure or functions (Ramírez et al., 2012; Zenobio, 2015). They are specifically applied for use in such activities as cleansing, toning, moisturizing, hydrating, exfoliating, conditioning, anointing, massaging, colouring/decorating, soothing, deodorizing, perfuming, and styling (Zenobio, 2015). Some products are used on a daily basis, whereas others are used less frequently (Biesterbos et al., 2013). When multiple products are used simultaneously, consumers may be exposed to the same substance through different sources and routes, also referred to as aggregate exposure (Lorenz et al., 2011).

The acronym PCPs can also be used to designate a group of chemical compounds used in the previously mentioned consumable products, such as parabens, synthetic musks, UV-filters, antiseptics, siloxanes, etc. They are of scientific and public concern, as newly recognized classes of environmental pollutants and are receiving considerable attention with respect to their environmental fate and toxicological properties over the last decade (Evgenidou et al., 2015). Therefore, they are considered emerging contaminants. PCPs enter the environment mainly through effluents discharged from wastewater treatment plants (WWTPs). In fact, these compounds are constantly introduced into the sewage system through human activities (bathing, showering, etc.) and most conventional WWTPs are not prepared to remove/degrade these contaminants. Therefore, they can be present in effluent wastewaters at relatively high concentrations (Golovko et al., 2014) and then discharged into the environment. Thus, PCPs have been detected in several environmental media such as surface water, sea water and sediments (Meffe and Bustamante, 2014; McClellan and Halden, 2010). Because of their continuous loading into the environment, most of these compounds are considered "pseudo-persistent" contaminants. In fact, various PCPs have been detected in wild fish inhabiting wastewater discharge areas, reflecting their constant exposure (Tanouea et al., 2014). Although in recent years more attention has been given to these classes of compounds, they still constitute an emerging issue due to the lack of sufficient information concerning their occurrence, fate and ecotoxicological effects in the environment (Alvarino et al., 2015; Oosterhuis et al., 2013). Similarly, they have been poorly studied in cosmetics and toiletries, one of the most important contamination sources.

As previously mentioned, organosiloxanes are considered PCPs compounds. In fact, it is known that they have been used in a variety of cosmetics and personal care products and many other consumer products (Wang et al., 2009), representing a large source of exposure for both humans and environment. However, data on product concentrations are very scarce. Concentrations of siloxanes in cosmetics and toiletries were first reported by Horii and Kannan (2008), but so far only one study was published in Europe (Dudzina et al., 2014). Their results were obtained form products acquired in the Netherlands and Switzerland markets, and measured siloxanes concentrations varied significantly across and within product categories (Dudzina et al., 2014).

2.1.1 Siloxanes

Siloxanes can be classified as inorganic siloxanes or organic siloxanes also called. By definition, inorganic siloxanes are considered saturated silicon-oxygen hydrides with unbranched or branched chains of alternating silicon (Si) and oxygen atoms (O), wherein each silicon atom is separated from its nearest silicon neighbours by single oxygen atoms (IUPAC, 1997). Organosiloxanes are designated as chemical compounds with a backbone of alternating atoms of silicon (Si) and oxygen (O), each atom bearing one or several organic groups (Lassen et al., 2005). The properties of these organosiloxanes depend on the length of the Si-O backbone, the chemical organic groups attached to the backbone and the presence of cross-links between the backbones.The silicon and oxygen atoms may be linked into cyclic or linear structures and they are classified as cyclic or linear siloxanes (Lassen et al., 2005). For the reader's sake, throughout this text "siloxane" should be understood as "organosiloxane" if nor otherwise stated.

Siloxanes are used in several industrial processes and consumer products. In the production of silicon-containing chemicals, they are released as a residue (Dewil et al., 2007). They are also used in paper-coatings, textiles (Dewil et al., 2007), paints (Lassen et al., 2005), lubricants, etc. and also to construct supramolecular architectures (Dewil et al., 2007). Due to their flexibility, resistance, to abrasion and heat they are also incorporated in plastics (Jovanovic et al., 2008) but are also used by the food industry as an oil substitute to create low-calorie alternative food products such as potato chips, salad dressings, and mayonnaise (SEHSC, 2009). Finally, siloxanes are widely incorporated in cosmetics and personal care products (Lassen et al., 2005; Jovanovic et al., 2008). In fact, siloxane polymers, which are solid polymers that are poorly soluble in water (Werme, 2010), may be used in bath products, eye makeup, lipstick, nail polish, as well as hair and skin care products (according to The Personal Care Products Council, 2014). In acordance with Jovanovic (2008), siloxanes are widely used in cosmetics industry due to their beneficial qualities, such as enhance skin feel, reduce in greasiness, and increase the absorption, leading to a silky shiny look.

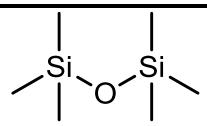
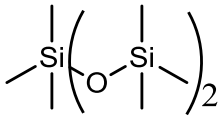
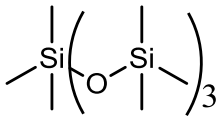
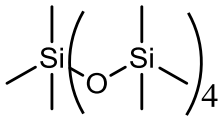
2.1.1.1 Linear siloxanes

Linear siloxanes are characterized by the functional side chain that they have attached to the Si-O backbone and the endgroups terminating the structure. The side groups may contain the same chemical groups as the structure or even several different side groups may be attached (Lassen et al., 2005). The presence of halogens in the side chains produces very stable polymers. Very high resistance to solvents can be obtained by using trifluoropropyl side chains, whereas lubricity at high temperatures is obtained by tetrachlorophenyl side groups. Typical endgroups are methyl, hydroxyl, vinyl or hydrogen (Biomonitoring Program, 2008).

The most important industrial polysiloxanes are linear polydimethylsiloxanes (PDMS), also called "dimethicone". In their most simple form they have methyl side-chains and methyl terminal groups (Lassen et al., 2005), i.e. consists of repeating units of $(CH_3)_2SiO$. PDMS can be found in a wide variety of industrial applications and consumer products, including cosmetic products and medical devices (Wang et al., 2009).

The most significant representatives of low molecular polydimethylsiloxanes, also known as linear volatile methylsiloxanes along with their chemical structure and some of their physicochemical properties are listed in Table 1.

Table 1: Chemical structure and characteristics of linear volatile methylsiloxanes (adapted from Kim et al. (2013)).

Compound Chemical Formula CAS No.	Chemical Structure	Molar mass ($g.mol^{-1}$)	Boiling Point (°C)	Water Solubility ($mg.L^{-1}$, 25 °C)	Vapor Pressure (mmHg, 25 °C)	Log K_{ow}
Hexamethyldisiloxane (L2) $C_6H_{18}Si_3O$ 1071-46-0		162	107	0.930	31.00	4.20
Octamethyltrisiloxane (L3) $C_8H_{24}Si_3O_2$ 107-51-7		236	153	0.035	3.90	4.80
Decamethyltetrasiloxane (L4) $C_{10}H_{30}Si_4O_3$ 141-62-8		301	194	0.007	0.55	5.40
Dodecamethylpentasiloxane (L5) $C_{12}H_{36}Si_5O_4$ 141-63-9		384	230	0.001	0.07	6.00

A lipophilic nature for all above presented siloxanes is noticeable as their log K_{ow} are always superior to 4 (Brebbia et al., 2011). Lipophilicity is proportional increasing with chain length. A similar trend can be for boiling points that range between 107 °C and 230 °C. In general, these compounds are considered volatile (boiling points less than 250 °C).

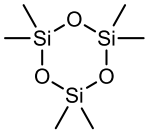
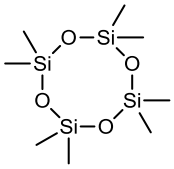
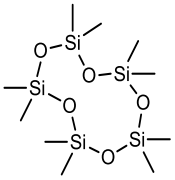
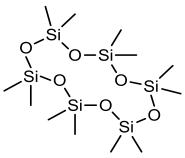
2.1.1.2 Cyclic siloxanes

In the cyclic siloxanes, the Si-O backbone forms a cyclic structure with two substituents attached to each silicone atom (Biomonitoring Program, 2008). These substituents are usually methyl groups. They are partly used as intermediates for the production of higher molecular weight linear siloxanes and partly used directly as fluids. Cyclic siloxanes with other functional groups, e.g. methylphenylcyclosiloxanes, are used for fewer applications. Cyclic volatile methylsiloxanes (cVMSs) are widely used in cosmetics products, in which mixtures of these compounds are known under the name “cyclomethicone” (Lassen et al., 2005).

cVMSs are arranged in a ring with two methyl groups attached to each silicon atom (Dudzina et al., 2014). The most used cyclic siloxanes are the hexamethylcyclotrisiloxane (D3, “D” refers to the dimethylsiloxane unit - $(CH_3)_2SiO$ - and the subscript refers to the number of silicon bonds) (Lu et al., 2011), octamethylcyclotetrasiloxane (D4), decamethylcyclopentasiloxane (D5) and dodecamethylcyclohexasiloxane (D6) (Biomonitoring Program, 2008). These compounds were found in higher amounts than linear siloxanes in various environmental matrices, including landfill gas, indoor and outdoor air, natural and sewage/industrial water and also in fish and mammal tissues (Wang et al., 2009). Typically the most widely used of the cyclic siloxanes, D4, can be found in several product types as for example paints, cleaning agents, dyes, fillers, polishes and adhesives. In most product groups, the total registered amount is, though, quite small (Lassen et al., 2005).

The most significant representatives of cyclic volatile methylsiloxanes, along with their chemical structure and some of their physicochemical properties are listed in Table 2.

Table 2: Chemical structure and characteristics of cyclic volatile methylsiloxanes (adapted from Kim et al. (2013)).

Compound Chemical Formula CAS No.	Chemical Structure	Molar mass ($g.mol^{-1}$)	Boiling Point (°C)	Water Solubility ($mg.L^{-1}$, 25 °C)	Vapor Pressure (mmHg, 25 °C)	Log K_{ow}
Hexamethylcyclotrisiloxane (D3) $C_6H_{18}Si_3O_3$ 541-05-9		222	134	1.560	10.00	4.47
Octamethylcyclotetrasiloxane (D4) $C_8H_{24}Si_4O_4$ 556-67-2		297	176	0.056	1.30	5.10
Decamethylcyclopentasiloxane (D5) $C_{10}H_{30}Si_5O_5$ 541-02-6		371	210	0.017	0.40	5.20
Dodecamethylcyclohexasiloxane (D6) $C_{12}H_{36}Si_6O_6$ 540-97-6		445	245[(a)]	0.005	0.02	6.33

(a) Value obtained from Lide (2007).

Observing the properties presented in Table 2, it is possible to conclude that, as verified for linear siloxanes, cyclic siloxanes present log K_{ow} values higher than 4, implying a lipophilic behaviour. The boiling points are between 134 °C and 245 °C, being D6 the less volatile compound. The value of water solubility is higher for D3 and lower for D6. They are highly insoluble in water, but hydrolytically stable and can be easily emulsified into most cosmetic preparations (Johnson et al., 2011).

2.2 Siloxanes in cosmetics

As it was referred before, siloxanes have been used in many consumer products, such as cosmetics and personal care products (Wang et al., 2009; Gouin et al., 2013). About 200 siloxanes and siloxane derivatives are registered in the inventory of ingredients used in cosmetic products compiled by the European Commission (INCI, 2000). Although they only account for a small part in the constitution of personal care products, once siloxanes represent a considerable source of exposure for both humans and environment. Dermal application of this kind of products is considered the most important human exposure route for siloxanes (Hanssen et al., 2013). However, other toiletries are applied by spraying, which increases the risk of exposure by inhalation. According to the data provided by the Cosmetic Toiletry and Fragrance Association (CTFA, 2006), which includes more than 75,000 cosmetics and toiletries, over 16% of these products contain siloxanes and D5 seems to be by far the most extensively used compound (EWG, 2012). In fact, both D5 and D6 have been known as high production volume (HPV) chemicals by the Organization for Economic Cooperation and Development (OECD, 2007). Shampoos, conditioners and stick deodorants seem to be those products, which have higher amounts of siloxanes.

So far, in Europe, there is no available legislation enforcing limits related to the maximum concentration levels of siloxanes in cosmetics or environmental matrices. However, the consumers of cosmetics and toiletries are protected by tough requirements provided in the Cosmetics Directive No. 1223/2009 (European Parliament, 2009) to guarantee the safety of cosmetics and by a strong commitment by manufacturers to utilize the best science and latest available research data to substantiate the safety of the product before it is placed on the market. In Canada, the Ministry of Environment published a notice requiring the preparation and implementation of pollution prevention plans in respect to D4 in industrial effuents, setting a maximum limit of 17.3 $\mu g.L^{-1}$ in those final effluents (Environment Canada, 2012).

High stability, physiologic inertness and good release are some of the characteristics of siloxanes. In fact, siloxanes and their derivatives function in the cosmetics as emollients, antifoaming, viscosity-controlling and antistatic agents, binders, film formers, emulsifying agents, humectants and also additives (Lassen et al., 2005). In particular, low molecular weight cyclic volatile methylsiloxanes (cVMSs) are widely employed as emollients and carrier solvents in personal care formulations, due to their distinctive physicochemical properties as volatility, hydrophobic nature, low surface tension, transparency, and lack of odour (Dudzina et al., 2014; Biesterbos et al., 2013). Once they are emollients and also act as conditioning agents, they help to provide a barrier on the surface of the skin or hair due to their hydrophobic nature related to the presence of the methyl groups, preventing dehydration. In fact, they are considered somehow occlusive agents, blocking water and preventing its escape from the surface of the skin (transepidermal water loss) or hair (Lees, 2011). Their low surface tension enhances their spread ability when compared to other organic emollients and their low heat of vaporization compared to water or ethanol prevents the cooling feel when they dry (Barel et al., 2014). So they promote a silky, smooth feeling on the skin and produce a better shine effect after application (SEHSC, 2009). As volatile carrier solvents they help to uniformly deliver active substances along the hair fibres or over the skin (Goddard and Gruber, 1999). Likewise, low molecular weight dimethicone fluids are also applied as emollients and together with cVMSs are also considered mild humectants (Lassen et al., 2005). These siloxanes have free electron pairs on the Si-O-Si bond (on the oxygen atoms), so they can form hydrogen bonds with proton donors as water molecules. In this way, there is a possibility to maintain the skin moist.

In Europe, cosmetic container labels must list all ingredients in the product formulation using identical terms across the whole European Union. These terms are based on the International Nomenclature for Cosmetics Ingredients (INCI). The packaging for some products (as liquid or solid soaps) makes it impractical to enclose a leaflet, label, tape or card, which occur for other products. The ingredients list for these products must appear on a notice in immediate proximity to the container in which the cosmetic product is exposed for sale (Dayan and Kromidas, 2011). The list of ingredients shall be set in descending order of weight of the ingredients at the time they were added to the cosmetic product. Ingredients with concentrations lower than 1% may be listed in any order after those in concentrations of more than 1% (European Parliament, 2009). When siloxanes are presented in cosmetic and toiletry labels, they are rarely listed by their chemical names, but by the generic names “dimethicone” or “cyclomethicone”, which represents the volatile linear and cyclic methylsiloxanes, respectively (Lassen et al., 2005).

The use of some types of siloxanes has caused concern among the scientific community due to their potential toxic behaviour to human health and environment (Horii and Kannan, 2008). Therefore, the incorporation of alternative compounds has been investigated for the products, in which the risk of human exposure and release to the ecosystems is considered high.

2.2.1 Toxicity

As mentioned before, siloxanes are organic ingredients used in a widespread variety of personal care products due to their high thermal stability and smooth texture. Therefore, for humans, the exposure to siloxanes can occur when PCPs, cosmetics and other consumer products are used (mainly dermal application or inhalation), and potentially could also occur through environmental exposure (Horii and Kannan, 2008). Although, they are incorporated in such kind of products, there are studies that report toxic effects produced by siloxanes in laboratory tests with animals (Quinn et al., 2007; Meeks et al., 2007; McKim et al., 2001a; Holson and Reynolds, 1997; Stump and Reynolds, 1997).

The most investigated compounds due to their toxicity are D4 and D5 (Lassen et al., 2005). Inhalation and oral exposures of D4 of rats resulted in impairment of fertility and reproductive failures (McKim et al., 2001a; Meeks, et al., 2007). According to Meeks et al. (2007), exposure to D4 on the day early to mating resulted in a significant decrease in fertility. Wider reproduction and fertility screening studies with inhaled D4 were performed in rats along earlier years (Holson and Reynolds, 1997; Stump and Reynolds, 1997). In all these performed studies, rats were exposed to whole body vapour inhalation to D4 at a concentration ranging from 70 to 700 ppm. Exposure was continuous for as early as 28 days before mating. The main conclusions noted in females exposure to D4 at 700 ppm were the decrease in the total number of pups born, number of uterine implantation sites, number of corpora lutea, and mean live litter size. These restrictions are all interrelated with a reduction in the number of ovulated eggs (characterized by the amount of corpora lutea), which will affect the number of implantation sites, and therefore the potential litter size. The mean live litter size in the 700 ppm exposure groups was consistently 60–70% of the control group. So, interferences with hormonal changes and tissues responses, during the rat oestrous cycle, may result in a decrease in number of corpora lutea and, as was referred, consequently, litter size. These effects were observed in the overall and fertilization phases, in which fertility was also significantly reduced, following an exposure to 700 ppm of D4 on the day early to mating. Analogous mechanisms control ovulation in both rats and humans. However, the control mechanisms for the timing and release of luteinizing hormone (LH) from the pituitary gland are quite different in rodents and in humans. The LH surge in humans is much broader than in rats, and the LH release, ovulation, and mating behaviour are all intimately linked and critically timed in rats, but not in humans. Thus, it is possible that the effect of a shift in humans do not have the same impact as in rats. While it is evident that suppression of the LH surge in rats can have dramatic consequences on the reproductive outcome, this mode-of-action may have little or no relevance for humans. Quinn et al. (2007) also demonstrated estrogenic effects of D4 by inhalation following *in vitro* exposures in rats. In this way, D4 was recommended for addition to the list of toxic substances under the Canadian Environmental Protection Act, once this study was performed in this area (Warner et al., 2010). Toxic effects of D6 are less known, but due to its similarity to other cyclic siloxanes it likely may have some toxic effects. McKim et al. (2001b) tried to understand if the exposure to a high concentration (700 ppm) of D4 vapours would result in an increase in hepatic hyperplasia over time. Therefore, the effect of inhalation exposure to D4 over several days was tested in rats. The lowest concentration tested (70 ppm), resulted in a significant growth in liver weight.

Few studies investigating the toxicity of these compounds after dermal exposure can be found in literature. Acute dermal toxicity of D4 was investigated in rats and rabbits. All performed tests (rats exposed to 2,400 $mg.kg_{bw}^{-1}$ and rabbits to 10,000 $mg.kg_{bw}^{-1}$) did not conducted to mortalities. However, the rabbits' tests produced ataxia, hyperactivity, eschar formation and burned areas on back of some animals (SCCS, 2010). Repeated dose (28 days) dermal toxicity studies were also performed in rabbits for the same compound. A No-

Observed-Effect Level (NOEL) of 960 mg.kg$_{bw}^{-1}$ was established for this study (SCCS, 2010). A sub-acute dermal toxicity study of D5 was also conducted in rats. In this study, rats were treated dermally with D5 under occlusive conditions at different dose levels (0, 200, 800, and 1600 mg.kg $_{bw}^{-1}$). Treatments were for 6 hours *per day*, 7 days *per week*, for 28 days. Under the test conditions, no significant toxicological effects were produced. Therefore, a NOEL of 1600 mg.kg$_{bw}^{-1}$ was established for the dermal application of D5 up to 4 weeks (SCCS, 2010). No information was found about the linear VMSs.

As there is some concern related to the presence of siloxanes in cosmetics and personal care products, and as they can be considered dangerous, alternatives with similar properties to siloxanes were developed as substitutes.

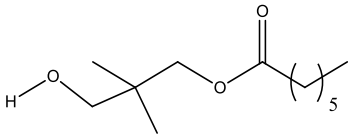
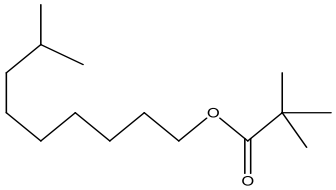
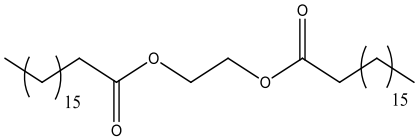
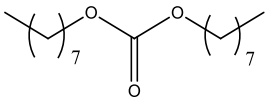
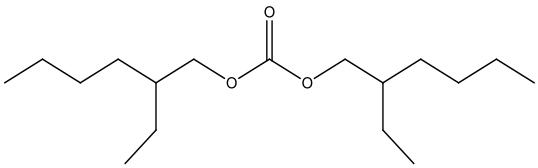
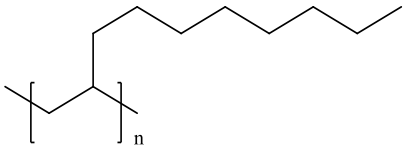
2.2.2 Alternatives to siloxanes in cosmetics products

As mentioned before, siloxanes have many functions in the several types of cosmetics products, imparting different properties to these consumer products. Because of this, it is not possible to find only one alternative for siloxanes. Therefore, alternatives must be very specific, as they have to comply with the special characteristics that the specific siloxane has in the given product (Lassen et al., 2005). It has been so far a difficulty, since the developed alternatives does not present all the siloxane properties. For example, siloxanes are incorporated in soaps and leave-on products, as skin lotions and creams, to provide the combination of "smooth and soft feeling" to the skin, without the greasy feel after application (Dudzina et al., 2014). The incorporation of cyclomethicone in this kind of products is difficult to substitute. However, dimethicone is easier to substitute, because the same properties can often be obtained with different types of vegetable oils (Lassen et al., 2005). Table 3 shows some alternatives to siloxanes used in cosmetic products.

Neopentylglycol heptanoate has the same good spreadability as dimethicone and can be used as solvent for other substances and emulsifiers (Weissermel and Arpe, 2003). Isodecyl neopentanoate can be used in leave-on products, conditioners and perhaps in shampoos and cream soaps. It has high spreadability, gives a soft feeling like cyclomethicone and can be used as solvent and emulsifier (Edser, 2015). On the other hand, glycol distearate is an alternative to siloxanes in different types of soaps. It gives a "milk-like" appearance to the products and contains wax that gives shine and smooth feeling to cream soaps, shower gels and shampoos. It can typically not directly substitute all the characteristics of dimethicone, cyclomethicone or other types of siloxanes, which often can give a more distinct feeling of softness, but it has similar properties (Bolzinger et al., 2007). Different vegetable oil components can be used in creams and lotions instead of siloxanes (Lassen et al., 2005). One particular example of these oils is dicaprylyl carbonate. It cannot directly substitute the properties of siloxanes used in lotions and creams, since the alternatives does not have the foam reducing effect that the siloxanes use to have. But apart from this, dicaprylyl carbonate can be used instead of cyclomethicone or dimethicone, and can add softness and spreadability to the products (Zhu et al., 2015). Another substitute of cyclomethicone in lotions and emulsions is the diethylhexyl carbonate, which is extremely spreadable and low-viscous ester oil. So it is possible to manufacture a product based on diethylhexyl carbonate that has almost the same qualities as those based on cyclomethicone (Lassen et al., 2005). Hydrogenated polydecene is an alternative to different basis mineral oil or paraffin oils. If a product contains both paraffin oils and cyclomethicone, this alternative can usually substitute both substances, as it can give some of the soft feeling on the skin and can easily be absorbed without greasing. Hydrogenated

polydecene can, however, not give the extra soft feeling that the siloxanes add to cosmetic leave-on products (Lassen et al., 2005).

Table 3: Identified alternatives to siloxanes used in cosmetic products (adapted from Lassen et al. (2005)).

Name	CAS no.	Chemical structure	Alternative to	Used in
Neopentylglycol heptanoate	N/A		Dimethicone	Conditioners and leave-on products
Isodecyl neopentanoate	60209-82-7		Cyclomethicone	Conditioners and leave-on products. Some shampoos and cream soaps.
Glycol distearate	627-83-8		Cyclomethicone and dimethicone in cream soaps*	Cream soaps
Different vegetable oil components (e.g. Dicaprylyl carbonate)	N/A		Dimethicone, cyclomethicone and other siloxanes*	Creams and lotions - do not have foam-reducing effect that some siloxanes have in creams and lotions
Diethylhexyl carbonate	N/A		Cyclomethicone	Lotions and emulsions
Hydrogenated poly(1-decene)	68037-01-4		Cyclomethicone in composition with paraffin oils	Leave-on products

*do not have exactly the same properties.

Most of the alternatives are competitive with siloxanes regarding their price. However, some of them cannot entirely replace the siloxanes' functions and properties. For instance, cyclomethicone and dimethicone in emulsions and creams are especially difficult to substitute, as the alternatives typically do not have a foam-reducing effect in the final products (Lassen et al., 2005). Therefore, research in this field must yet be further developed.

2.3 Environmental exposure to siloxanes

It is expected that human wastes are the main source of environmental contamination. In fact, siloxanes are incorporated in products with a high utilization rate and, therefore, they are continuously introduced into the environment through the sewer systems (Pierce, 2004; Dewil et al., 2007) or solid waste deposition in landfills (Pierce, 2004).

Due to their properties, namely lipophilicity and bioaccumulative potential, these compounds are only partially biodegradable and therefore, when they reach the conventional WWTPs are not completely removed (Bletsou et al., 2013). Therefore, effluents and sludge are the primary contamination mediums. However, due to their relatively lower solubility in water, they do not tend to accumulate in higher concentrations in the water phase, but are preferentially adsorbed on sludge (Neyens et al., 2004). For this reason, the disposal of sludge to terrestrial environment through landfill or agricultural application also contributes to the release of siloxanes into the environment. It is also important to notice that most siloxanes are semi-volatile compounds and, for that reason they also disperse into the atmosphere, where they are decomposed (Raich-Montiu et al., 2014).

Several studies have reported the presence of siloxanes in different environmental compartments, including air (MacLeod et al., 2013; Kierkegaard and McLachlan, 201; Yucuis, 2013; Pieri et al., 2013; Genualdi et al., 2011), river water (Companioni et al., 2012; Whelan et al., 2010; Sparham et al., 2008), seawater (Hong et al., 2014), sediments (Zhang et al., 2011), soils (Lockwood, 2015; Kozerski et al., 2014; Brunete, et al., 2010), biota such as fish, crustacean and seafood (Jia et al., 2015; Lockwood, 2015; Kierkegaard and McLachlan, 2013) and even in human samples, such as breast milk (Dirtu et al., 2012) and human plasma (Xu et al., 2015; Hanssen et al., 2013). The concentration of siloxanes in air samples were in the range of $ng.m^{-3}$, varying from 29 to 280 $ng.m^{-3}$ in outdoor air (Yucuis et al., 2013) and from 18 (Pieri et al., 2013) to 2200 $ng.m^{-3}$ (Yucuis et al., 2013) in indoor air. In water samples the concentration of siloxanes are in the range of $ng.L^{-1}$. River water usually contains values in the range of 0.09 to 3.94 $ng.L^{-1}$ for linear siloxanes and 22.2-58.5 $ng.L^{-1}$ for cyclic siloxanes (Companioni et al., 2012). In the specific case of D5, the concentration levels are generally lowest, ranging between <10 and 29 $ng.L^{-1}$ (Sparham et al., 2008). Seawater usually contains values below 46.1 $ng.L^{-1}$ (Hong et al., 2014). For sediments and soils, the detected concentration range for cyclic siloxanes (D4 to D7) was between 602 and 2360 $ng.g^{-1}$ and for linear siloxanes (L4 to L16) was between 98 and 3310 $ng.g^{-1}$ (Zhang et al., 2011). In biota samples, D5 was detected in higher concentrations than other cyclic siloxanes, usually with average values below 31.7 $ng.g^{-1}$ (Kaj et al., 2005), and the linear (L2 to L16) were detected with values between 0.3 to 0.5 $ng.g^{-1}$ (Kaj et al., 2005). On the other hand, for human samples the highest values detected were between 2.69 and 12.7 $ng.mL^{-1}$ (Hanssen et al., 2013). In almost every case, cyclic siloxanes were found in higher concentrations than linear.

2.4 Analytical methods for the determination of siloxanes in cosmetics

Some analytical methodologies have been developed for the determination of linear and cyclic siloxanes in cosmetics and personal care products, most of them based on gas chromatography-mass spectrometry (GC-MS) analysis. These analytical methods include, not only the sample extraction and cleanup, but also the instrumental analysis.

2.4.1 Extraction techniques

This section presents a brief description of the most usual extraction techniques for the determination of siloxanes in cosmetics and personal care products (such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE)). The extraction technique used in this work, Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) will be explained in more detail.

2.4.1.1 Liquid-liquid extraction (LLE)

Liquid-liquid extraction (LLE) is the most common technique for the extraction of compounds from aqueous-based samples (Schuur et al., 2011). In this method, a liquid sample interacts with an immiscible solvent, in which the analyte of interest is more soluble. This dispersion creates a high interfacial area, which increases the extraction rates. In fact, LLE is based on principles of mass transfer (Jiao et al., 2015). The target analyte is distributed between the two immiscible phases, according to its solubility (Viegas et al., 2007). Normally, one phase is aqueous (often the heavier phase) and the other is an organic solvent (the lighter phase). The base of the extraction process is that more polar hydrophilic compounds have a higher affinity to aqueous (polar) phase and the more non-polar hydrophobic compounds prefer organic solvent (Dean, 2009).

2.4.1.2 Solid-phase extraction (SPE)

Solid-phase extraction (SPE) is an increasingly useful sample preparation technique. With SPE, many of the problems associated with LLE can be prevented, such as incomplete phase separation, the use of expensive and breakable specialty glassware, and disposal of large quantities of organic solvents (Somsen and Jong, 2002). SPE is usually more efficient and faster than LLE, yields quantitative extractions that are easy to perform, and can be automated (Lacaze et al., 2007).

SPE is more often used to prepare liquid samples and extract semivolatile or non-volatile analytes, but can also be used with solids that are pre-extracted into solvents. In fact, SPE combines sample extraction, pre-concentration and cleanup (Yebra et al., 2008). The major advantage of this technique, when compared with other extraction techniques, is the availability of different type of sorbents. SPE also offers more advantages such as reduction of solvent usage, reduction or elimination of matrix interferences, prevention of contamination and *in situ* coupling with many detectors (Jalbani et al., 2014; Ezoddin et al., 2014). Consequently, in recent years, SPE has been successfully used for separation and sensitive determination, mainly in water samples (Jalbani et al., 2014). This method is one of the various techniques available to an analyst to bridge the gap between the sample collection and the analysis step. Filtration, homogenization, precipitation, chemical reaction, solvent exchange, concentration, matrix removal, solubilisation, are just a few of the available tools that may be used individually or in combination to get the sample into a form compatible with the analytical instrument required for analysis. SPE is seldom used without other auxiliary sample preparation steps, such as dilution or pH adjustment (Simpson, 2000).

2.4.1.3 Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS)

The Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) method is based on the work published by Anastasiades et al. (2003). This technique involves a sample preparation and extraction step, followed by an extract clean-up (Sapozhnikova and Lehotay, 2013). The sample is weighted and internal standards (if used) are added at this point. Then, the process involves two simple phases (Figure 1): the homogenized samples are extracted with an organic solvent in an ultrasound bath and salts are added to ensure partitioning; then the extract suffers a clean-up process through a dispersive solid-phase extraction (dSPE) (Anastassiades, 2006).

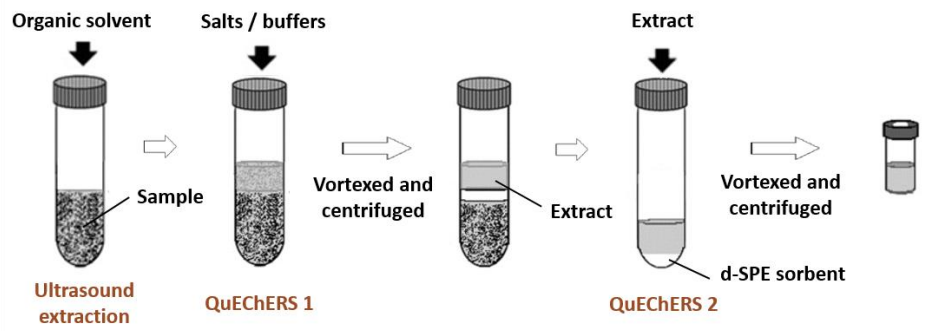


Figure 1: Simplified diagram representative of the QuEChERS procedure (adapted from Ribeiro et al., 2014).

Different salts, acids, and buffers (e.g. $MgSO_4$, Na_2SO_4, NaCl and $NaCH_3COO$) may be added to enhance the separation and extraction efficiency of the analytes to the organic phase and protect sensitive analytes, preventing their degradation by maintaining an optimal pH. In the second step, appropriate sorbents (e.g. C_{18}, PSA, graphitized carbon) are used to remove the undesired sample components. The last step consists in the sample analysis. Here the samples may be pH adjusted or solvent-exchanged prior to analysis by either GC-MS or LC-MS (Picó, 2008; Zhao et al., 2014; Park et al., 2011).

In fact, QuEChERS is a user-friendly technique that has been replacing the conventional LLE and SPE and also became very versatile, being adapted to a wide range of substances and matrices. These matrices include, not only food products as animal-based products (meat, fish, kidney, chicken, milk and honey), cereals and grains, wines, juices, fruit and vegetables (Zhao et al., 2014), but also environmental (Groz et al., 2014; Brondi et al., 2011) and cosmetic products (Homem et al., 2013). QuEChERS sample preparation approach has emerged as an alternative method for analyte extraction because it requires little organic solvent and usually conducts to low limits of detection (Aysal et al., 2007). In fact, a driving force in the growth of QuEChERS is the emerging need to determine trace amounts of analytes (Picó, 2008). This technique presents as main advantages the speed (short extraction/clean-up time), low cost and minimum handing of extracts, when compared with the previously techniques, and wide applicability (Aysal et al., 2007; Ribeiro et al., 2014).

In the next sub-chapter a brief description of the GC-MS, the analytical equipment used in this project is given.

2.4.2 Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectrometry (GC-MS) is the most ubiquitous analytical technique for the identification and quantification of organic substances in complex matrices, which provides a high sensitivity and specificity (Courant et al., 2007). The gas chromatograph-mass spectrometer is crucial in the fields of environmental science, health care, medical and biological research, health safety, the flavour and fragrances industry, food safety, packaging, and many others (Agostino et al., 2015).

This method combines two microanalytical techniques. The gas chromatograph separates the different components of a mixture in time, and the mass spectrometer provides information that aids in the structural identification of each component (Abian, 1999). So this combination has several advantages as it separates

components of a complex mixture so that mass spectra of individual compounds can be obtained for qualitative purposes. Mass spectrometry techniques that require gas-phase analytes are ideally suited to GC-MS because sample volatility is a requirement of GC (Sparkman et al., 2011). A scheme of a typical simple GC-MS is represented in Figure 2.

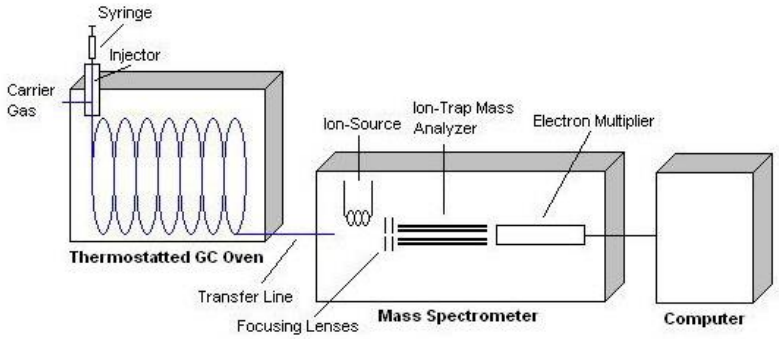


Figure 2: Scheme of a typical simple GC-MS (adapted from Sparkman et al., 2011).

A number of options are available for GC inject systems, which allow the introduction of the sample into the equipment (Courant et al., 2007). The choice of optimum sample introduction strategy depends on the concentration range of target analytes, their physicochemical properties and on the occurrence of matrix co-extracts present in the sample. The most widely used injector is the split/splitless injector (Hinshaw et al., 2009). In the split mode, the injected sample is vaporized into the stream of carrier gas, and a small portion of the sample and solvent is directed onto the head of the GC column. The split mode is used for samples that are fairly concentrated. The injector temperature should be high enough to volatilize the analytes and the initial column temperature should be just below the temperature, at which the first compound elutes (Douglas, 2011). In the splitless mode, the sample is injected with the splitter vent closed. After a specific time, the splitter vent is opened to purge solvent from the injector. The analytes present in the sample are deposited onto the head of the column and most of the volatile solvent is vented. With splitless injection, the injector temperature should be high enough to volatilize all of the analytes (Douglas, 2011). After injection, the sample is directed introduced into the column.

There are two types of columns used in GC, packed and open tubular, also known as capillary column. Capillary columns are frequently used due to their separation efficiency. The column is placed in a thermostatic oven, which allows the control of the temperature along the time. The boiling point of the solvent and analytes defines the optimal column temperature and the choice of the most suitable stationary phase is essential for a good separation of the different analytes in a sample (Douglas, 2011; Dauner and Sauer, 2000). This way, when the sample contains analytes with wide range of boiling points, a temperature program of is employed. The stationary phase must reveal some affinity for the analytes, if contrary there would be no retention and the components would leave the column during column dead time (Skoog et al., 2007). A carrier gas (usually helium) is used to transport the sample species through the column.

Then, the analytes reach the mass spectrometer detector. The analytes are first ionized and fragmented by an ion source. The ion source can be either considered a hard ionization (electron ionization, EI) or a soft ionization (chemical ionization, CI) source. In the case of EI, a bunch of electrons passes through the gas-phase sample and the electron that passes in near proximity to a neutral analyte molecule can knock off another electron, resulting in a positively charged ion. The ionization process can either produce a molecular ion,

which will have the same molecular weight of the starting analyte, or it can produce a fragment ion, which corresponds to a smaller piece of the analyte molecule. Most MS use electrons with an energy of 70 electron volts (eV) for EI. Decreasing the electron energy can reduce fragmentation, but it also reduces the number of ions formed (Dunnivant and Ginsbach, 2008). On the other hand, the soft ionization uses ion-molecule reactions to produce ions from the analyte. The process begins when a reagent gas such as methane, isobutane or ammonia is ionized by electron ionization. A high reagent gas pressure results in ion-molecule reactions between the reagent gas ions and reagent gas neutrals. Some of the products of these ion-molecule reactions can react with the analyte molecules to produce analyte ions (Háková et al., 2015).

Quadrupoles and ion traps are the most used mass analysers. Quadrupoles consists in a filter of four parallel rods arranged in the form of a square. Each pair of opposite rods is connected with the other. The ion trap, the mass analyser that is going to be used in this project, has electrodes like a single quadrupole, but wrapped into a circle, as it is showed in Figure 3. There are, thus, two convex end-cap electrodes, in which the ions enter and leave through the end-caps (Hill, 2009).

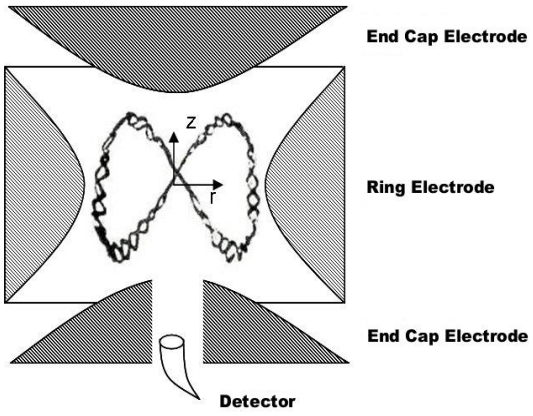


Figure 3: Diagram of an ion trap analyser (adapted from Hill, 2009).

A voltage is applied between the ring electrode and the two end-caps, so an ion inside the trap will find itself in a potential well. As the ion get closer to the negative electrode, the field changes, and this electrode switch to a positive charge. In fact, the field is constantly rotated, which results in a circular motion of the ions in the trap. Due to inertia, the largest ones tend to end up in the middle of the trap, where the potential is never changed. The smaller ions will always be dragged around a bit more in the field (Courant et al., 2007).

For the detection of ion signals emerging from the mass analyser an electron multiplier is used. Its task is amplify and finally detect every ion of the selected mass passed by the mass filter. Therefore, the performance of the electron multiplier may have a major effect on the overall performance of the mass spectrometer (Quemet et al., 2014). The process that allows an electron multiplier to operate is called secondary electron emission. When a charged or neutral particle, ion or electron strikes its surface, it causes secondary electrons to be released from atoms in the surface layer of the electron multiplier. The number of secondary electrons released depends on the type of incident primary particle, its angle, energy and characteristics of the incident surface (Quemet et al., 2014; March, 2000).

The data is stored as a tree-dimensional block with three axes: intensity, time and mass/charge ratio (m/z). The representation of signal intensity vs. GC retention time is designated as chromatogram, while signal intensity vs. mass/charge ratio is designated by mass spectrum. Mass spectrometer data can be done in full-

scan or selected ion storage (SIS). A full-scan ion chromatogram is a summation of the intensities of all mass fragments at a given time, while in SIS mode, only the selected *m*/*z* ranges are detected by the instrument during the analysis (Sparkman et al., 2011). Nowadays, GC-MS systems are usually operated by a computer, which controls the physical parameters of the system (e.g. temperatures, gas flow, etc.) and records the data generated during a run - chromatograms and mass spectra (Sparkman et al., 2011).

GC-MS is a versatile technique to separate, quantify and identify unknown volatile or semi-volatile organic compounds. Due to its high sensitivity and resolving power, this technique allows the analysis of very complex mixtures (Sparkman et al., 2011). However, the use of GC-MS is limited to analytes that are not only volatile, but also thermally stable. In fact, compounds that exist only in the gas phase at temperatures below 100 ºC cannot be separated and ionized using techniques other than GC-MS (Agostino et al., 2015).

Instead of the use of GC-MS for the analysis of siloxanes, it is also possible to use gas chromatography-flame ionization detector (GC-FID). In that case, the compounds that elute from the chromatographic column dissolved in the carrier gas pass through FID detector, where they will undergo pyrolysis at the flame temperature, producing ions and electrons which conduct electricity through the flame (Jawaid et al., 2014). This method can be used for the analysis of volatile organic compounds (VOC) or even semi-volatile compounds.

In this project, the GC-MS is used instead of GC-FID because the latter presents some restrictions that could result in limitations to the work. In fact, the retention time of a compound cannot be adjudicative or confirmatory of the specificity of that compound, meaning that a peak at a given retention time is not a unique qualitative measure (Clark et al., 2012). The information provided by GC-MS, the mass spectrum, is essential for the identity confirmation of the target compounds.

3 State of the Art

There are only a few studies that have been developed to quantify and determine siloxanes in cosmetics and toiletries, most of them based on GC-MS analysis. Plausibly, before quantification it is necessary to use an extraction technique. Therefore, it is expected that according to the nature of the matrices, one extraction method can be more efficient than others. However, the already performed studies compiled in Table 4, show that some authors used the same extraction method for distinct toiletries, although some conditions have been adapted.

The extraction methods used more frequently to determine siloxanes in cosmetics and toiletries matrices are liquid-liquid extraction (LLE) and ultrasound extraction (USE) followed by solid-phase extraction (SPE). In LLE and USE, the most common used extraction solvents were methanol (MeOH) (Wang et al., 2009; Dudzina et al., 2014), hexane (Hex) (Horii and Kannan, 2008; Wang et al., 2009; Dudzina et al., 2014), acetone (Acet) (Horii and Kannan, 2008; Wang et al., 2009) and ethyl acetate (EA) (Horii and Kannan, 2008), used according to the type of matix. These solvents have different polarities, in which methanol and acetone present higher polarities than ethyl acetate and hexane. The referred solvents have a good affinity to mid-low polar compounds as siloxanes. On the other hand, SPE was also used after ultrasound extraction in two studies performed by Dudzina et al. (2014) and Lu et al. (2011) as a cleanup methodology. Samples were purified by passage through a silica gel cartridge and compounds eluted with hexane and dichloromethane. Once more, the use of a slightly polar sorbents is explained by the mid-low polarity of siloxanes. Analysing Table 4 it is possible to conclude that similar recovery values were obtained for the different tested methodologies, varying between 73% (Lu et al., 2011) and 115% (Dudzina et al., 2014). Lower limits of quantification (5-22 $ng.g^{-1}$) were achieved using the methodology based on USE-SPE followed by GC-FID proposed by Lu et al. (2011). A more detailed analysis can be found in the text below.

The first study to report the concentrations of organosiloxanes (cyclic siloxanes) in a range of 76 PCPs (skin lotions, body washes, hair care products and cosmetics) was made by Horii and Kannan (2008). Concentration of cyclic siloxanes in consumer products ranged from <LOQ to 9.38 $mg.g^{-1}$ for D4, <LOQ to 81.80 $mg.g^{-1}$ for D5, <LOQ to 43.10 $mg.g^{-1}$ for D6, <LOQ to 0.85 $mg.g^{-1}$ for D7, and also from <LOQ to 73 $mg.g^{-1}$ for the linear siloxanes (L4 to L14), using GC-MS. Skin lotion samples had the highest total concentrations of linear siloxanes. Among linear siloxanes, mean concentration of L11 was the highest (0.34 $mg.g^{-1}$), followed by L10, L12 and L9, and lowest for L4 (0.03 $mg.g^{-1}$). On the other hand, for cyclic siloxanes, mean concentration of D5 was the highest (13.6 $mg.g^{-1}$) for cosmetics such as lipstick and liquid foundation.

The study performed by Wang et al. (2009) showed levels of cVMSs in 252 cosmetics and PCPs present in the Canadian market, with the purpose to understand the sources and also extent of potential dermal exposure of consumers to these compounds. Analysed samples were divided into six categories, including fragrances, hair care products, deodorants and antiperspirants, nail polishes, skin lotions and a variety of baby products such as oils, lotions, shampoos, and diaper creams. Because of the diverse physical properties of the products, different extraction solvents were employed. For fragrances, hair products (except hair gels), deodorants and baby products (except lotions and shampoos), samples were extracted with hexane by USE. On the other hand, hair gels, skin lotions and shampoos were extracted with a mixture of methanol and hexane. A different approach was used for nail polishes, where samples were first extracted with acetone and then with hexane. This diversity in used solvents was due to the wide variety of the studied sample and the need

to solubilize them in the extraction solvent to enhance cVMSs extraction efficiency. cVMSs were measured using GC coupled with MS. Eventually, D5 was the most often detected cVMS (in 14.3% of the analysed samples), as it had been also observed by Horii and Kannan (2008). Maximum levels of D5 was detected in an antiperspirant (683 $mg.g^{-1}$), D6 in a baby diaper cream (98 $mg.g^{-1}$), D4 in a body lotion (11 $mg.g^{-1}$) and D3 also in diaper cream (0.45 $mg.g^{-1}$). The detection frequency of cVMSs in baby products was low (only three baby products contained cVMSs). Two of three positive samples were baby diaper creams, one of which contained relatively high levels of all cVMSs (0.45 $mg.g^{-1}$ of D3, 0.52 $mg.g^{-1}$ of D4, 150 $mg.g^{-1}$ of D5 and 97.7 $mg.g^{-1}$ of D6). The Canadian government reported that inhalation exposure to these cyclic siloxanes make up to more than 99% of total daily consumption of cVMSs, with upper-bounding exposure estimated at 3.5 $mg.day^{-1}$ for D4, 5.6 $mg.day^{-1}$ for D5 and 2.3 $mg.day^{-1}$ for D6 (EC/HC, 2009). Despite of the limits and doubts in the estimation of the dermal exposure to cVMSs through the use of cosmetics and PCPs, it cannot be considered to be insignificant based on the fact that high levels of cVMSs are present in some of these products.

Lu et al. (2011) studied the concentrations and characteristics of siloxanes in selected PCP marketed in China, to allow the determination of dermal exposure of these compounds through the usage of PCPs. In this way, 15 siloxanes, including four cyclic siloxanes (D4-D7) and 11 linear siloxanes (L4-L14) were analysed in 158 PCPs. Samples were extracted by USE, using hexane and a mixture of hexane/ethyl acetate as extraction solvent, promoting the transition of the analytes from the semi-solid phase to the liquid phase. The concentrated samples were purified by solid-phase extraction (SPE) containing silica gel topped with sodium sulphate and elution was performed using with a mixture of hexane and dichloromethane. Obtained extracts were analysed with GC-FID. This latter instrumental method was chosen because of its advantages over the more commonly used method, GC-MS, like cost effectiveness and less resource consumption (Pacchiarotta et al., 2010). The concentrations obtained were considerably lower for all the four cVMSs. Siloxanes were detected in 88% of the samples analysed, in which cyclic siloxanes were found in 71% and D5 and D6 were the most frequently detected compounds. Toothpastes presented the lowest frequency of detection for cyclic siloxanes, with concentration levels lower than the limits of quantification, but all hair care products contained these compounds. D4 (87%), D5 (91%) and D7 (89%) were the most detected cyclic siloxanes, with a mean concentration of 0.013 $mg.g^{-1}$, 0.054 $mg.g^{-1}$ and 0.009 $mg.g^{-1}$, respectively. Highest levels of D6 were found in cosmetic products (90%) with a mean concentration of 0.067 $mg.g^{-1}$, and hair care products (89%) with 0.016 $mg.g^{-1}$, and on the other hand, the total of linear siloxanes (L4-L14) were found in concentrations around 6.87 $mg.g^{-1}$ into cosmetic products and 0.015 $mg.g^{-1}$ into hair care products. The profiles of both types of siloxanes found in these samples are different from what was reported in the U.S. and in Japan (Horii and Kannan, 2008), in which PCPs contained lower rates of occurrence of linear siloxanes (only 33%) than cyclic siloxanes. Linear siloxanes were found to be predominant in all categories of products, except for hair care products, in which total cyclic siloxanes accounted for 88% of total siloxanes.

The objective of the study performed by Dudzina et al. (2014) was to measure the concentrations of cyclic siloxanes in cosmetics and PCPs that are currently available in the European market. Levels of D4, D5, and D6 in 51 selected cosmetics and PCPs, such as hair care products, deodorants and antiperspirants, skin lotions, sun care products, cosmetics and toothpastes were assessed. Depending on the product type, the sample extraction was slightly adapted. For skin lotions and creams the samples were extracted with methanol, followed by hexane in order to confirm complete extraction of target chemicals. GC-FID was chosen for routine analyses due to the same reasons presented above. This methodology resulted in relatively high values of LOQs for D4, D5 and D6 (715, 670 and 721 $ng.g^{-1}$, respectively). All samples contained detectable amounts of at least one of the three cVMSs studied, although D5 was the most prevalent compound, being

detected in 47 out of 51 products. It was found in higher concentrations in antiperspirants with a mean concentration of 356 $mg.g^{-1}$. Both D5 and D6 were detected in higher concentrations compared to D4 in each subcategory, with D5 predominated in almost all the products. The mean and median concentrations of D5 for all the products that had detectable amounts are 60.5 $mg.g^{-1}$ and 25.7 $mg.g^{-1}$, respectively. The concentrations of D6 are in a lower range with a mean of 7.0 $mg.g^{-1}$ and a median of 0.6 $mg.g^{-1}$. D4 was detected at least once in every subcategory, with the exception of hand and sunscreen creams. In this work, the daily exposure to D4 and D5 was estimated using the Ford dermal exposure model (Ford, 1998), which means that assuming the value of dermal absorption rate of 0.5% and 0.04% for D4 and D5, respectively (Jovanovic et al., 2008), the maximum dermal doses available for systemic absorption would be 0.054 and 0.49 $mg.capita^{-1}.day^{-1}$, respectively. Relatively high dermal exposure to both cVMSs occurs from the use of face creams with 27% (D4 and D5), body lotions with 1.0% (D4) and 18% (D5), and liquid foundations with 2.0% (D4) and 9.0% (D5) of the total dermal dose, respectively. Overall, the results were in agreement with those obtained by Horii and Kannan (2008) and Wang et al. (2009), who report that D5 and D6 are the most used cyclic siloxanes in cosmetics and PCPs.

In conclusion, cyclic siloxanes are the mostly detected siloxanes in personal care products. In this way, D5 was the compound found at highest concentration levels (1.11 to 683 $mg.g^{-1}$), followed by D4 (0.37 to 151 $mg.g^{-1}$). The presence of siloxanes in personal care products excelled in shampoos, conditioners and deodorants/antiperspirants.

It is important to notice that the product concentrations of siloxanes determined in those studies can also serve as essential input data for environmental fate modelling, exposure assessment, and validation of cVMS emissions based on the monitoring of the environmental media (Buser et al., 2013).

Table 4: Overview on analytical methods for determinations of siloxanes in different personal care products.

Matrix	Country	Analytes	Extraction/Cleanup method	Instrumental method	% REC	LOD ($ng.g^{-1}$)	LOQ ($ng.g^{-1}$)	C ($mg.g^{-1}$)	References
-Hair care products -Deodorants and antiperspirants -Skin lotions -Sun care products -Cosmetics -Toothpaste	Netherlands and Switzerland	D4	0.1-0.5 g sample USE (3 mL MeOH + 3 mL Hex for skin lotions and creams and 3 mL Hex for the other products; 2 x 3 mL Hex; 15 min) SPE (0.7 g silica gel; elution with 5 mL DCM/Hex 1:9)	GC-FID (random checks with GC-MS)	75.7±17.6	350	715	<LOQ-5.00	(Dudzina et al., 2014)
		D5			88.5±17.8	328	670	<LOQ-356.00	
		D6			114.5±20.2	353	721	<LOQ-151.00	
-Toothpaste -Hair care products -Body washes -Toilet soaps -Skin lotions -Makeup products	China	D4	0.3-0.5 g sample USE (2 x 5 mL Hex; 5 mL EA/Hex 1:1; 20 min) SPE (0.5 g silica gel; elution with 6 mL Hex + 5 mL DCM)	GC-FID	72.8±10.2	na	17	<LOQ-0.07	(Lu et al., 2011)
		D5			80.2±6.7		5	<LOQ-1.11	
		D6			80.1±7.4		22	<LOQ-0.37	
		L4-L14			na		5-7	<LOQ-52.60	
-Fragrances -Hair care products -Deodorants and antiperspirants -Nail polishes -Skin lotions -Body products	Canada	D3	0.2-0.4 g sample LLE (4 mL Hex - for fragrances, hair products (except hair gels), deodorants and baby products (except lotions and shampoos); 4 mL MeOH + 4 mL Hex - For hair gels, lotions, skin cleansers, and shampoos; 1 mL Acet + 4 mL Hex - nail polishes; 1200 rpm; 15 min)	GC-MS	74.5	12000	na	0.12-0.45	(Wang et al., 2009)
		D4			91.7	8000		0.01-11.00	
		D5			99.0			0.02-683.00	
		D6			102.3			0.01-97.70	
-Hair care products -Body washes -Skin lotions -Cosmetics	USA and Japan	D4	0.1-0.3 g sample LLE (4 x 3 mL EA/Hex 1:1; 15 min)	GC-MS	87.0±5.4	na	351	<LOQ-9.38	(Horii and Kannan, 2008)
		D5			87.0±9.4		387	<LOQ-81.80	
		D6			90.0±10		333	<LOQ-43.10	
		D7			na		415	<LOQ-0.85	
		L4-L14			na		59 for L2-L5 117 for L6-L9 294 for L10-L14	<LOQ-73.00	

LLE – Liquid-liquid extraction; LOD - limit of detection; LOQ - limit of quantification; na - not available; REC - mean recovery; SPE - solid-phase extraction; USE - ultrasound extraction; Solvent abbreviations: Acet – acetone; DCM – dichloromethane; EA - ethyl acetate; Hex – hexane; MeOH – methanol; (*) – mean value.

Since there are only a few studies related to the development and determination of siloxanes in cosmetics and toiletries, the evaluation of the quantities of siloxanes released "down-the-drain" was also purposed. In this way, a research about the concentrations of both cyclic and linear siloxanes in wastewaters was performed (Table 5), emphasising the levels detected in the influents. It is also important to notice that this literature review is not intended to be exhaustive, but only an indicator of the levels and mass loads of these pollutants in wastewater treatment plants.

To the author's best knowledge, the study performed by Egmond et al. (2013) was the first study reporting estimates of the concentration of cVMS in wastewater. They attempted to accurately measure the concentration of D4, D5 and D6 in untreated and final effluent in order to estimate the *per capita* loading of cVMS into a sewage treatment plant (STP). The analytical method used in this study was headspace-gas chromatography-mass spectrometry (HS-GC/MS). This is a partition-based method (headspace) that has numerous advantages including high sample throughput, low limits of quantification and the potential to quantify dissolved and total concentrations of siloxanes in the same sample. In this way, concentrations between 2.0 and 23.5 $\mu g.L^{-1}$ for D6, 5.6 and 35.5 $\mu g.L^{-1}$ for D5 and <0.2 $\mu g.L^{-1}$ for D4 were detected in the wastewater influent. On the other hand, for effluent samples, the concentrations were around 0.1 $\mu g.L^{-1}$ for D6, 0.3 $\mu g.L^{-1}$ for D5 and <0.01 $\mu g.L^{-1}$ for D4. The average influent mass loads for D6 were lower than for D5, with values about 0.0013 $g.capita^{-1}.day^{-1}$ and for D5 about 0.0027 $g.capita^{-1}.day^{-1}$.

The study reported by Xu et al. (2013) determined levels of four cyclic (D3, D4 and D5) and two linear siloxanes (L3 and L4) in aqueous and sludge samples from a wastewater treatment plant (WWTP). The analytical method used in this study included solid-phase microextraction (SPME) followed by GC-MS analysis. L3 was not detected in any of the aqueous samples analysed, while L4 was barely found in the influent. On the contrary, cyclic siloxanes, which are mainly used in cosmetics and toiletries, had higher detection frequencies and concentration levels. D3, D4, D5 and D6 were detected in all samples with concentrations between 0.48-0.59 $\mu g.L^{-1}$, 2.42-2.89 $\mu g.L^{-1}$, 3.04-3.29 $\mu g.L^{-1}$ and 2.20-2.56 $\mu g.L^{-1}$ in influent, and nd-0.12 $\mu g.L^{-1}$, 0.25-0.55 $\mu g.L^{-1}$, 0.50-1.00 $\mu g.L^{-1}$ and 0.52-0.95 $\mu g.L^{-1}$ in the final effluent, respectively. Influent mass loads were estimated for all the cyclic siloxanes analysed: 0.0003 $g.capita^{-1}.day^{-1}$ for D3, 0.0013 $g.capita^{-1}.day^{-1}$ for D4, 0.0016 $g.capita^{-1}.day^{-1}$ for D5, and 0.0012 $g.capita^{-1}.day^{-1}$ for D6.

The purpose of the study performed by Wang et al. (2013) was to analyse the influent and effluent from several Canadian WWTPs, as well as nearby ambient water and sediment. So, to extract the aqueous samples a membrane-assisted solvent extraction technology (MASE) was used. Since cVMS are highly volatile compounds, the water samples were processed without filtration to avoid losses and contamination from handling and processing. The concentrations of D4, D5 and D6 in influent varied in the range 0.28-6.69 $\mu g.L^{-1}$, 7.75-135.00 $\mu g.L^{-1}$, and 1.53-26.90 $\mu g.L^{-1}$, respectively. D5 was the dominant cVMS compound in almost all the samples, followed by D6 and D4. Most influents had concentrations lower than 60 $\mu g.L^{-1}$ and the effluent concentrations of D4, D5 and D6 were in the range <0.009-0.045 $\mu g.L^{-1}$, <0.027-1.56 $\mu g.L^{-1}$, and <0.022-0.093 $\mu g.L^{-1}$, respectively. The highest concentration of D5 in effluent was 1.56 $\mu g.L^{-1}$ and the second highest concentration was 1.31 $\mu g.L^{-1}$ for D5.

The study performed by Bletsou et al. (2013) using traditional liquid-liquid extraction showed that all cyclic (D3-D7) and linear siloxanes (L4-L14) analysed were detected in influent and effluent samples. The total average concentration of siloxanes in influent was 20.3 $\mu g.L^{-1}$, with cyclic siloxanes (75%) contributing more to this value than linear siloxanes. D5 and D6 were the major compounds found in influents at mean

concentrations of 2.60 and 1.83 μg.L^{-1}, respectively. These cyclic siloxanes are the predominant compounds present in cosmetics and toiletries, being considered the responsible sources of these siloxanes in WWTPs. Concentrations of siloxanes in final effluents were lower than the concentrations found in influents. The average total concentration of siloxanes in effluents was 3.58 μg.L^{-1}. In effluents, cyclic siloxanes were also the predominant compounds present in these samples, accounting for 59% of the total concentrations determined. On the other hand, linear siloxanes accounted for 41% of the total concentrations in effluents. D5 was the cyclic siloxane found in major concentration levels in effluents (1.79 μg.L^{-1}), whereas the concentrations of individual linear siloxanes were <0.03 μg.L^{-1}. The average mass influent load were estimated for all the cyclic and linear siloxanes analysed. The mean mass loads for the total cyclic siloxanes were 0.0011 g.capita^{-1}.day^{-1}, while for the total linear siloxanes were 0.0031 g.capita^{-1}.day^{-1}. D5 and L11 presented the higher values, about 0.0005 g.capita^{-1}.day^{-1} and 0.0010 g.capita^{-1}.day^{-1} respectively.

Sanchís et al. (2013) studied the concentration levels of linear (L3-L5) and cyclic siloxanes (D3-D5) in influents and effluents of 17 WWTPs from Spain (Catalonia) using LLE. Siloxanes were detected in all analysed wastewater samples, although a significant reduction was observed during the wastewater treatment processes. D5 was the predominant compound, with a mean concentration of 8.825 μg.L^{-1} in the influent and 0.545 μg.L^{-1} in effluent. For most of the samples the median value of the results for D5 in wastewater presented was comparable with the few available data in freshwater studies (Sparham et al., 2008).

Cortada et al. (2014) also determined linear and cyclic siloxanes in wastewater samples. They found by preliminary experiments that conventional liquid-liquid extraction of siloxanes in wastewater samples produced emulsion problems. This emulsion problem was not verified when liquid-liquid microextraction (LLME) was carried out. However, ultrasound-assisted dispersive liquid-liquid microextraction (USA-DLLME) was the method chosen for this work, as an advantageous mode of LLME. In this method, the used extraction solvent must fulfil the following requirements: preferably have a higher density than water, low solubility in this medium, high extraction capability of the target analytes and be easily dispersed in water during sonication (Vidal et al., 2007; Cortada et al., 2011). It should also have good chromatography behaviour (Cortada et al., 2011). In this specific case, chlorobenzene was selected. Different factors can affect the extraction yield in the USA-DLLME procedure and in most cases they could be correlated, so in this way, a multivariate approach was used for the optimization (Regueiro et al., 2008). This technique is considered faster, cheaper and easier to handle than other methods, as LLME. In this study, D4 and D5 were the cyclic siloxanes with higher concentrations in influent samples, with 3.6±0.8 μg.L^{-1} and 4.8±1.0 μg.L^{-1}, respectively. In effluent samples, the siloxanes with higher concentrations were D4 and D6 at 2.2±0.5 μg.L^{-1} and 1.2±0.3 μg.L^{-1}, respectively. In relation to the linear siloxanes, all the analysed compounds obtained concentrations lower than the limits of detection.

The study performed by Wang, et al. (2015a) using MASE, allowed the determination of the concentration profile of VMSs in influent and effluent samples from a Chinese WWTP. Here, only D4, D5 and D6 were detected in both types of samples analysed. The mean concentrations of D4, D5 and D6 in influent and effluent were 1.59 μg.L^{-1} and 23.5 μg.L^{-1}, 9.60 μg.L^{-1} and 0.02 μg.L^{-1}, and 0.14 μg.L^{-1} and 0.06 μg.L^{-1}, respectively. Generally, the mean concentration (1.05 μg.L^{-1}) of cVMSs in influent water from this region is lower than those in Greece with 5.14 μg.L^{-1} (Bletsou et al., 2013), Spain with 9.20 μg.L^{-1} (Cortada et al., 2014), and Canada with 44 μg.L^{-1} (Wang et al., 2013). Final effluent concentrations were lower than those register in influent samples, which were 0.097 μg.L^{-1}, 0.156 μg.L^{-1}, and 0.090 μg.L^{-1} for D4, D5 and D6, respectively. The concentration of cVMSs in waste sludge samples were also estimated in this study. The average influent mass

loads were estimated for all the cyclic siloxanes analysed. The predictable values for D4, D5 and D6 were 0.0682 mg.capita^{-1}.day^{-1}, 0.0715 mg.capita^{-1}.day^{-1} and 0.0548 mg.capita^{-1}.day^{-1}, respectively.

Wang et al. (2015b) also performed a similar study to determine cVMS in a municipal WWTP from Canada. They verified that concentrations of the selected siloxanes fluctuated in influents from 0.17 to 1.13 mg.L^{-1} for D4, 3.47 to 19.3 mg.L^{-1} for D5, and 0.45 to 3.87 mg.L^{-1} for D6. Final effluent concentrations were lower than those found in the influent and ranged from <0.01 to 0.03 mg.L^{-1}, 0.19-0.24 mg.L^{-1} and 0.01-0.02 mg.L^{-1} for D4, D5, and D6, respectively. D5 was the dominant cVMS in the influent and effluent samples. Mass inputs were also estimated in this study. Average values of 0.0004 g.capita^{-1}day^{-1} for D4, 0.0072 g.capita^{-1}day^{-1} for D5, and 0.0010 g.capita^{-1}day^{-1} for D6 were achieved.

In conclusion, there are only few studies related to the presence of siloxanes in wastewater. Cyclic siloxanes are more frequently studied than linear. When both are presented in this type of matrix, cyclic siloxanes are detected in higher concentrations and, normally, D5 is the predominant, as found in cosmetics and personal care products. For the studies in wastewater, the concentrations of siloxanes in influents are higher than in effluents, which leads us to believe that these compounds are removed with some efficiency from the water line. As mentioned before, during and after use of toiletries and personal care products, some siloxanes will be discharged to wastewater. But especially D5, is expected to volatilise to the atmosphere (≈ 90%) (Brooke et al., 2005), although not all the emissions end in this way. “Rinse-off” products are likely to result in emissions to wastewater, where absorption and volatilisation play significant and competing roles in the removal of cVMS during sewage treatment (Egmond et al., 2013).

According to all the information provided above, it was important to create and develop this project in order to evaluate and determine the concentrations of siloxanes in cosmetics and personal care products (main source of contamination) once this data is scarce. In fact, in Portugal there are no studies related to this theme, and in the rest of Europe only one study was performed.

Table 5: Overview on analytical methods for determinations of siloxanes in wastewater samples.

Country	Analytes	Extraction/Cleanup method	Instrumental method	% REC	LOD (ng.L^{-1})	LOQ (ng.L^{-1})	Concentration in influent (µg.L^{-1})	Concentration in effluent (µg.L^{-1})	References
Canada	D4	100 mL sample MASE (0.5 mL pentane, 28.5 °C, 60 min, 325 rpm)	GC-MS	102±10	9	na	0.17 - 1.13	0.01 - 0.03	Wang et al., 2015b
	D5			104±16	27	na	3.47 - 19.3	0.18 - 0.24	
	D6			107±12	22	na	0.45 - 3.87	0.01 - 0.02	
China	L3	100 mL sample MASE (0.5 mL Hex, 25 °C, 60 min, 200 rpm)	GC-MS	93	na	82	<LOQ	<LOQ	Wang et al., 2015a
	L4			97	na	90	<LOQ	<LOQ	
	L5			92	na	91	<LOQ	<LOQ	
	D3			72	na	23	<LOQ	<LOQ	
	D4			91	na	27	0.225 - 0.521	0.050 - 0.181	
	D5			92	na	32	0.301 - 0.439	0.106 - 0.185	
	D6			94	na	19	0.256 - 0.354	0.045 - 0.150	
Spain	L2	13 mL sample USE-DLLME (13 µL chlorobenzene, 2 min USE)	GC-MS	71-84	6	20	≤LOD	≤LOD	Cortada et al., 2014
	L4			73-93	3	10	≤LOD	≤LOD	
	L5			71-86	20	70	≤LOD	≤LOD	
	D3			71-86	400	1300	≤LOD	≤LOD	
	D4			71-82	2	7	3.6±0.8	2.2±0.5	
	D5			72-99	3	10	4.8±1.0	≤LOD	
	D6			73-92	30	100	≤LOD	1.2±0.3	
Spain	L3	500 mL sample LLE (3 x 250 mL Hex)	GC-MS	50.2 - 75.4	0.4±0.1	1.2±0.2	nd - 0.006	nd - < LOQ	Sanchís et al., 2013
	L4			58.1 - 108.9	0.4±0.1	1.4±0.4	nd - 0.015	nd - < LOQ	
	L5			75.4 - 112.0	0.10±0.02	0.5±0.1	0.054 -1.307	nd - 0.029	
	D3			40.3 - 74.6	7.4±2.0	15.0±4.1	nd - 0.724	nd - 0.322	
	D4			55.0 - 75.7	13.0±4.8	26±11	<LOQ - 1.089	nd - 0.476	
	D5			93.5 - 114.6	3.2± 0.4	6.3±0.8	- 24.484	0.042 - 3.587	

GC-MS - gas chromatography-mass spectrometry; LLE – liquid-liquid extraction; LOD - limit of detection; LOQ - limit of quantification; MASE – membrane-assisted solvent extraction; na - not available; nd - not detected; REC - mean recovery; USE-DLLME – ultrasound-assisted dispersive liquid-liquid microextraction; Solvent abbreviations: Hex – hexane

Table 5: Overview on analytical methods for determinations of siloxanes in wastewater samples (cont.).

Country	Analytes	Extraction/Cleanup method	Instrumental method	% REC	LOD (ng.L^{-1})	LOQ (ng.L^{-1})	Concentration in influent (µg.L^{-1})	Concentration in effluent (µg.L^{-1})	References
Greece	D3	100 mL sample LLE (50 mL Hex, 25 mL Hex: DCM (1:1), 25 mL Hex: EA (1:1))	GC-MS	61±4	0.10	0.30	0.114 - 0.183	0.095 - 0.256	Bletsou et al., 2013
	D4			81±17	0.03	0.11	0.099 - 0.187	0.103 - 0.197	
	D5			87±13	0.06	0.18	0.544 - 5.36	0.125 - 6.02	
	D6			105±12	0.20	0.60	1.16 - 3.19	0.002 - 0.059	
	D7			134±14	0.22	0.66	0.294 - 0.579	0.009 - 0.016	
	L3			82±9	2.00	7.00	<LOD	<LOD - 0.005	
	L4			101±15	4.00	12.00	<LOD - 0.148	<LOD - 0.099	
	L5			108±6	0.20	0.60	0.010 - 0.067	0.0007 - 0.012	
	L6			80±13	0.41	1.20	0.079 - 0.968	0.011 - 0.163	
	L7			91±13	0.39	1.20	0.093 - 1.98	0.020 - 0.310	
	L8			95±14	0.65	2.10	0.440 - 3.14	0.019 - 0.343	
	L9			100±16	0.84	2.90	0.469 - 4.43	0.027 - 0.484	
	L10			115±20	1.90	6.10	1.33 - 4.89	0.030 - 0.500	
	L11			118±19	6.10	18.00	1.20 - 7.91	0.042 - 0.634	
	L12			111±16	13.00	40.00	0.438 - 1.57	0.012 - 0.085	
	L13			107±15	8.10	24.00	0.137 - 0.726	0.007 - 0.035	
	L14			101±8	7.20	22.00	0.045 - 0.210	<LOD - 0.013	
Canada	D4	100 mL sample MASE (polyethylene membrane, 0.5 mL pentane, 28.5 °C, 60 min, 325 rpm)	GC-MS	100±21	9	31	0.282 - 6.69	<LOD - 0.045	Wang et al., 2013
	D5			103±21	27	90	7.750 - 135.00	<LOD - 1.560	
	D6			107±29	22	73	153.0 - 26.90	<LOD - 0.093	

GC-MS - gas chromatography-mass spectrometry; LLE – liquid-liquid extraction; LOD - limit of detection; LOQ - limit of quantification; MASE – membrane assisted solvent extraction; REC - mean recovery; Solvent abbreviations: DCM – dichloromethane; EA – ethyl acetate; Hex – hexane

Table 5: Overview on analytical methods for determinations of siloxanes in wastewater samples (cont.).

Country	Analytes	Extraction/Cleanup method	Instrumental method	% REC	LOD (ng.L^{-1})	LOQ (ng.L^{-1})	Concentration in influent (µg.L^{-1})	Concentration in effluent (µg.L^{-1})	References
China	D3	40 mL sample HS-SPME (PDMS/DVB fibre, 24 °C, 45 min, NaCl)	GC-MS	84 - 89[(a)] 83 - 85[(b)]	10.2	na	0.480 - 0.590	ND - 0.120	Xu et al., 2013
	D4			90 - 93[(a)] 89 - 86[(b)]	6.3	na	2.420 - 2.890	0.250 - 0.550	
	D5			86 - 89[(a)] 87 - 92[(b)]	4.7	na	3.040 - 3.290	0.500 - 1.000	
	D6			83 - 86[(a)] 87 -94[(b)]	3.1	na	2.200 - 2.560	0.520 - 0.960	
	L3			78 - 89[(a)] 82 - 90[(b)]	4.9	na	nd	nd	
	L4			85 - 92[(a)] 83 - 86[(b)]	4.8	na	nd - 0.070	nd	
UK	D4	15 mL sample Sample dilution	HS-GC/MS	74±3[(a)] 114±4[(b)]	na	0.2	<0.200	<0.01	Egmond et al., 2013
	D5			84±10[(a)] 76±14[(b)]	na	0.2	5.550 - 35.500	0.305 - 0.347	
	D6			67±11[(a)] 110±13[(b)]	na	0.2	2.010 - 23.500	0.071 - 0.117	

[(a)] influent concentration (µg.L^{-1}); [(b)] effluent concentration (µg.L^{-1})

DVB – divinylbenzene; HS-GC/MS – headspace-gas chromatography-mass spectrometry; HS-SPME – headspace extraction – solid-phase microextraction; LOD - limit of detection; LOQ - limit of quantification; na - not available; nd - not detected; PDMS – polydimethylsiloxane

4 Technical Description

4.1 Chemicals and materials

Eight siloxanes (four cyclic and four linear) were investigated in this study. Individual linear (L2-L5) and cyclic (D3-D6) volatile siloxanes and also the internal standard used, tetrakis(trimethylsilyloxy)silane (M4Q), were purchased from Sigma-Aldrich (St. Louis, MO, USA) with a purity >97%. For the QuEChERS preparation, anhydrous magnesium sulphate ($MgSO_4$) and sodium acetate ($NaCH_3COO$) were also obtained from Sigma-Aldrich (St. Louis, MO, USA), while PSA bonded silica and C18 from Supelco (Bellefonte, PA, USA). The $MgSO_4$ was baked at 450 ℃ overnight before use. n-Hexane (analytical grade) were purchased from VWR (Fontenay-sous-Bois, France). Helium (99.999%), used in the GC-MS system, and nitrogen (99.999%) for solvent evaporation, were supplied by Air Liquide (Maia, Portugal).

4.2 Standards preparation

For each siloxane, including the internal standard M4Q, individual stock solutions were prepared in hexane at 1.0 $g.L^{-1}$. A 15.0 $mg.L^{-1}$ final mix stock solution containing all cyclic and linear siloxanes was prepared by diluting appropriate amounts in hexane. An intermediate solution of M4Q was prepared at 150 $mg.L^{-1}$ in hexane and from this, a final stock solution with a concentration level of 75.0 $mg.L^{-1}$. The calibration standards (0.005 - 2.50 $mg.L^{-1}$) were prepared also in hexane from the final mix stock solution of siloxanes and M4Q (final concentration of 500 $\mu g.L^{-1}$). A standard solution of M4Q at 5.0 $mg.L^{-1}$ in hexane was also prepared from the initial stock solution (1.0 $g.L^{-1}$) and was used during the samples extraction. All solutions were protected from the light and preserved at -20 ℃.

4.3 QuEChERS preparation

For the analysis of each sample, two different QuEChERS were prepared. The first one contained 800 mg of anhydrous $MgSO_4$ and 750 mg of $NaCH_3COO$. The $MgSO_4$ is a drying agent and therefore, it is used to decrease the water amount, promoting the migration of siloxanes to the organic phase. The $NaCH_3COO$ acts as a buffer, maintaining an optimal pH value (pH 5.0-5.5), avoiding the degradation of the target compounds. It is also used to increase even more the aqueous phase polarity, enhancing the extraction of siloxanes. The second QuEChERS contained 60 mg of $MgSO_4$ to remove the remaining water, 60 mg of primary and secondary amine exchange sorbent (PSA) and 30 mg of octadecyl-silica (C_{18}). The PSA is used to remove sugars, fatty acids, organic acids, lipids, and some pigments existing in the extract, while C_{18} is used to remove long chain fatty acid compounds, sterols and other non-polar interferences (Hubschmann, 2015).

4.4 Samples

In this study, 136 cosmetics and toiletries were purchased from retail stores in Oporto (Portugal), according to the best selling brands in this region. The samples were divided into different categories according to their overall composition: moisturizers (n = 29), toothpastes (n = 12), toilet soaps (n = 15), shower gels (n = 23), deodorants/antiperspirants (n = 12), shaving products (n = 11) and hair care products (n = 34). Samples were kept in their original containers at room temperature until analysis.

4.5 Sample extraction

A total of 500 mg of each sample was weighed into disposable polypropylene conical tubes and 100 µL of a 5.0 $mg.L^{-1}$ in hexane of M4Q (internal standard) was added. The extraction solvent (hexane) was added (3 mL) and the samples were vortexed and sonicated for 3 and 10 min, respectively. After this, the first QuEChERS was added to the sample and then, the mixture was vortexed for 3 min and centrifuged for 10 min at 3700 rpm. The supernatant was removed from the first QuEChERS and transferred to a tube containing the second QuEChERS. The mixture was vortexed and centrifuged again under the same conditions as before, and the supernatant was transferred to an amber glass vial. The extract was dried under a gentle stream of nitrogen, reconstituted with 1.0 mL of hexane and analysed by GC-MS. Whenever necessary, extracts were further diluted to an appropriate volume and reanalysed.

4.6 Instrumental analysis

The extracted samples were analysed using a Varian Ion Trap GC-MS system. The mass spectrometer was operated in the electron ionization (EI) mode (70 eV). The separation was obtained at a constant flow of helium (1.0 $mL.min^{-1}$), using a Varian CP-SIL 8-CB capillary column (50 m × 0.25 mm, 0.12 µm). The oven temperature was programmed as follows: 35 °C hold for 5 min, raised at 6 $°C.min^{-1}$ to 155 °C and then 20 $°C.min^{-1}$ to 300 °C (hold for 2.75 min) - total time of analysis of 35 minutes. Injection (1 µL) was in split mode, with the split ratio of 5. Temperatures of manifold, ion trap, transfer line and injector were maintained at 50, 250, 250 and 200 °C, respectively. The filament emission current was 50 µA. For quantitative analysis of target compounds, selected ion storage (SIS) mode was applied. Table 6 shows the retention times and the quantifier and qualifier ions used for the SIS detection.

Table 6: Quantifier/qualifier ions of each siloxane analysed by GC-MS and respective retention time.

Compound	Retention time (min)	Quantifier Ions (*m/z*)	Qualifier Ions (*m/z*)
L2	5.75	147	73, 131
D3	9.54	207	133, 191
L3	11.58	221	73, 133
D4	15.13	281	193, 265
L4	17.14	207	73, 191, 295
D5	19.37	267	73, 355
M4Q	20.18	281	73, 147, 369
L5	21.61	281	73, 148, 369
D6	23.49	341	73, 325, 429

4.7 Quality assurance/Quality control

Due to the extensive use of siloxanes, special precautions were taken into account in order to prevent samples contamination. During this study, analysts avoided the use of personal care products such as hand creams and lotions and switched gloves whenever they changed sample. Procedural blanks were analysed with every extraction batch. Blank values were subtracted for all of the concentrations reported. Chromatographic blanks were also performed, but no memory effects were observed.

4.8 Waste management

The waste generated in the present work consisted of organic solutions containing hexane with trace amounts of siloxanes and also residues of personal care products and sorbents that were used during the extraction and clean-up ($MgSO_4$, $NaCH_3COO$, PSA and C_{18}). All residues were collected in proper closed containers, correctly labelled, and stored protected from light and ignition sources for further treatment by the Environmental Management System of FEUP - EcoFEUP.

5 Results and Discussion

5.1 Analytical method performance

Previous work performed in the laboratory in which this project was settled, has allowed the implementation of a technique by QuEChERS to determine the concentration profile of synthetic musks in personal care products (Homem et al., 2013). To the author's best knowledge, so far this type of methodology has never been applied to the analysis of siloxanes in toiletries and personal care products. Therefore, the aim of this project, as mentioned before, was to develop a methodology for the determination of siloxanes in personal care products by QuEChERS followed by GC-MS analysis. Thus, due to the physicochemical similarity between these two classes of emerging pollutants, it was decided to use the previously optimized method as a starting point, making adjustments, whenever necessary.

5.1.1 Adjustment of the methodology

Some changes were performed to the instrumental method proposed by Homem et al. (2013). Individual standards of each siloxane (500 µg.L^{-1} in hexane) were injected on the GC-MS in full-scan mode (m/z = 50 - 1000), using the previously mentioned methodology. With these analyses, the mass spectra of the selected organosiloxanes were acquired and the elution profile established. After that, a mix standard of siloxanes (500 µg.L^{-1} in hexane) was also injected on the GC-MS in full-scan mode, monitoring the same range of masses. At this point, the temperature programme used to separate the musks presented in personal care products (Figure 4) was tested. Injection was in splitless mode, with the split valve closed for 5 min and the injector port at 200 °C.

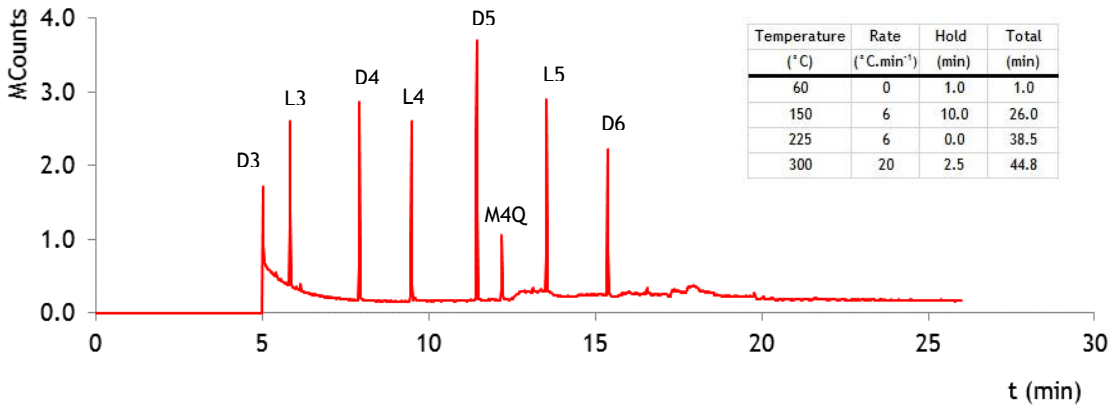


Figure 4: Chromatogram of a 500 µg.L^{-1} mix siloxane standard in hexane and respective temperature programme used.

As L2 is the most volatile target compound (b.p. = 107 °C), it elutes too early using the proposed temperature programme, not being clearly detected in the chromatogram (L2 seems to co-elute with the solvent). In fact, an excessively high column temperature results in very short retention time, but also in a very poor separation. Due to the high volatility of L2, it seems to have a poor interaction with the stationary phase, co-eluting with the solvent. Furthermore, the duration of the chromatographic run seems to be too

long, since the compounds' peaks arise during the first 20 minutes of the chromatogram. Because of that, it was necessary to adjust the temperature programme (Figure 5). The new values of the temperature programme were chosen according to the information available in the literature and the temperature of the injector was maintained at 200 °C.

Temperature (°C)	Rate (°C.min^{-1})	Hold (min)	Total (min)
35	0	5.0	5
155	6	0.0	25
300	20	2.8	35

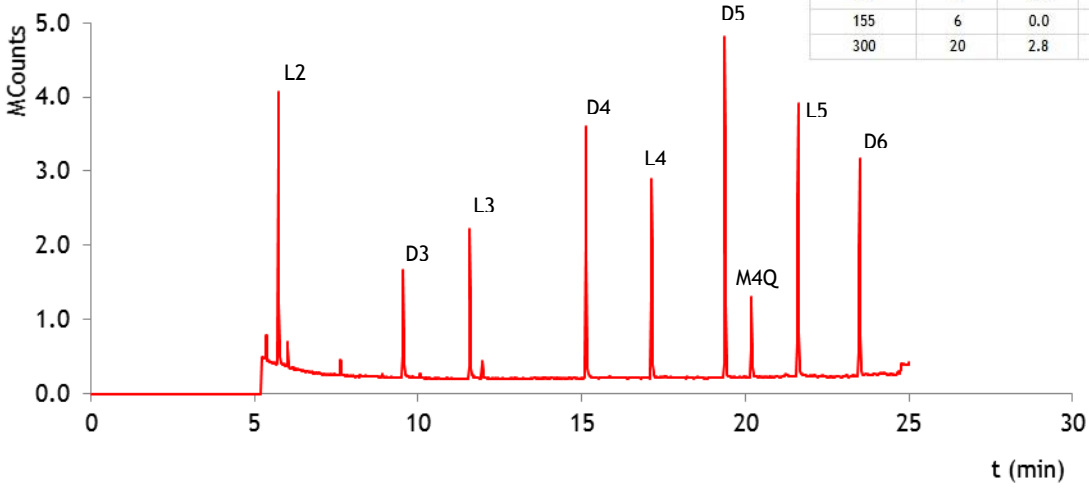


Figure 5: Chromatogram of a 500 µg.L^{-1} mix siloxane standard in hexane and the new temperature programme used.

The initial temperature and hold time generally affect the resolution of early eluting peaks. Therefore, in the new proposed programme, the initial temperature was decreased to 35 °C (the lowest practical oven temperature that could be used) and the temperature *plateau* increased for 5 minutes. These initial adjustments allowed the separation of L2 and the solvent (hexane). Then, a temperature gradient was incorporated (from 35 to 155 °C at 6 °C.min^{-1}). An increase in temperature will increase the vapour pressure of the analytes, reducing its interaction with the stationary phase of the capillary column. For that reason, during a temperature ramp, separation of compounds is mainly based on differences of boiling temperatures/vapour pressures. A temperature program usually shortens the required time for late-eluting analytes to pass through the column, while allowing the adequate separation of analytes that elute early in the analysis. With these adjustments all siloxanes were visible and presented a good separation (till 25 minutes). After the last compound was eluted (D6), which occurred at 23.5 minutes, the heating rate was significantly increased to 20 °C.min^{-1} in order to achieve the clean-up temperature sooner.

After this, the injection mode was also tested. In this case, the injection was adjusted to the split mode 5:1. As previously mentioned, in the splitless mode all the vaporised sample is transferred to the head of the chromatographic column, while in split mode only a fraction is transported onto the column. The remaining portion of the vaporised sample is removed from the injection port via the split vent line. This kind of injection should only be used when sample concentrations are high enough to allow a portion of the sample to be discarded during the injection process, which is expected in the determination of siloxanes in personal care products. In fact, the injection of the 500 µg.L^{-1} standard in split mode, allowed a better definition of the peaks in the chromatogram.

After suitable conditions were found, the 500 µg.L^{-1} mix standard of siloxanes was once more injected in full scan to determine the final retention times of the target compounds as well as the different mass

spectra. Then, acquisition was performed in selected ion storage mode (SIS). In SIS mode, the mass spectrometer only scans over a very small mass range and, for that reason, the sensitivity is usually enhanced. Furthermore, the undesirable ions are filtered, and selectivity is also greatly enhanced, being an additional tool to eliminate difficult matrix interferences. With the information collected in the full scan injection, retention time windows and ion ranges were defined to be used in SIS mode (Table 7).

Table 7: Definition of the ion ranges for each siloxane in SIS mode.

Compound	Ion range (*m/z*)	t_r (min)
L2	72-75, 130-133, 146-151	5.20-7.50
D3	132-135, 190-194, 206-213	7.50-10.50
L3	72-75, 131-134, 220-224	10.50-13.50
D4	190-195, 264-269, 280-284	13.50-16.00
L4	72-75, 190-194, 206-212, 294-297	16.00-18.00
D5	72-75, 266-270, 354-358	18.00-19.80
M4Q, L5	72-75, 146-151, 280-284, 368-372	19.80-22.50
D6	72-75, 324-328, 340-345, 428-432	22.50-25.50

The comparison between these two modes is presented in Table 8, in relation to the values of the peak areas.

Table 8: Comparison between areas in full-scan and SIS mode.

Compound	Full-scan	SIS
	Area (UA)	
L2	7.767×10^6	1.227×10^7
D3	3.767×10^6	5.256×10^6
L3	4.693×10^6	6.042×10^6
D4	6.460×10^6	9.089×10^6
L4	5.670×10^6	8.676×10^6
D5	9.629×10^6	1.307×10^7
M4Q	2.811×10^6	3.469×10^6
L5	7.709×10^6	1.024×10^7
D6	6.303×10^6	9.002×10^6

As can be concluded from Table 8, the values of the peak areas are higher when SIS mode is used due to increased selectivity of this data acquisition method, as mentioned before. In figure 6 is presented a chromatogram with all compounds analysed in SIS mode.

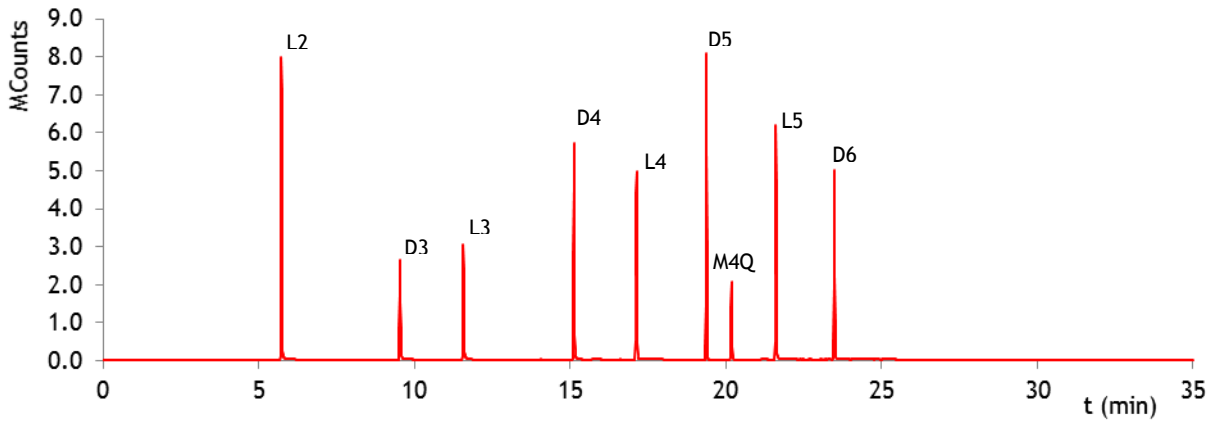


Figure 6: SIS mode chromatogram of a 500 µg.L^{-1}of a standard solution of siloxanes in hexane.

The extraction method applied in this work was also based on the technique performed by Homem et al. (2013), for the extraction of synthetic musks from personal care products (QuEChERS). This consists in a sample preparation and extraction step, followed by a clean-up. First of all, the sample is weighted and the internal standard is added. Then, the process involves two simple phases: the homogenized samples are extracted with an organic solvent in an ultrasound bath, and salts are added to ensure a partitioning; then the extract suffers a clean-up process through a dispersive solid-phase extraction (dSPE). The extraction conditions used were similar to the proposed by Homem et al. (2013), except for the extracting solvent. Since siloxanes present less polarity than musks, hexane was used instead. This modification was tested with spiked skin moisturizer samples (water-in-oil emulsion) because it was considered the more complex matrix within the range of products chosen. The resulting chromatograms are in Appendix 1.

5.1.2 Method validation

To evaluate the quality of the implemented analytical method, validation tests were performed. These allowed the determination of a set of statistical parameters that made possible the evaluation of characteristics of the method, as linearity, limits of detection and quantification, precision and accuracy and also the global uncertainty.

Quantification parameters

Calibration curves were constructed by direct injection of ten calibration standards in hexane containing all siloxanes at different levels (0.005 to 2.50 mg.L^{-1}). In fact, concentrations were correlated with the response factors (RF = $A_{siloxane}/A_{internal\ standard}$), using M4Q as internal standard (500 µg.L^{-1}). In Figure 7 is represented the calibration curve of D5, one of the most used siloxane in cosmetic formulations, and the respective confidence limits. In Appendix 2 all the calibration curves for the siloxanes analysed in this project are presented.

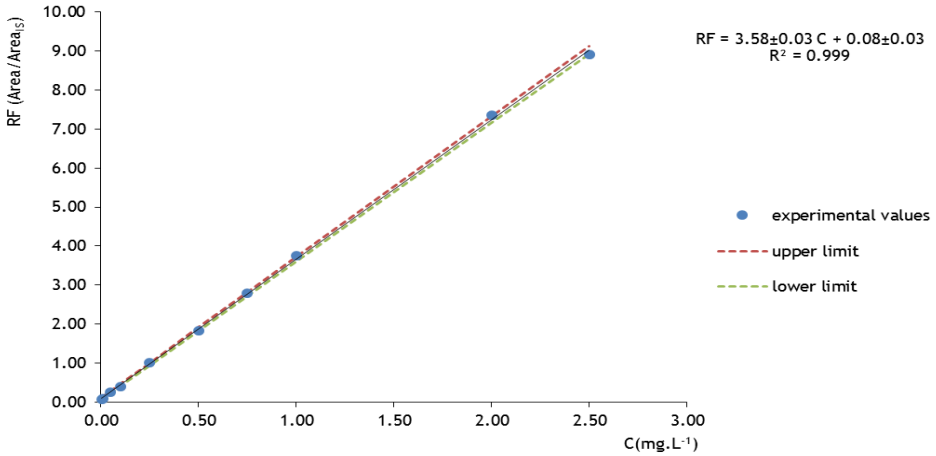


Figure 7: Calibration curve for D5 and the respective confidence limits.

The limits of detection (LOD) were calculated based on a signal/noise ratio (S/N) equal to 3, while the limits of quantification (LOQ) were obtained for an S/N = 10. The main results are presented in Table 9.

Table 9: Linearity range, detection and quantification limits for each compound studied.

Compound	Linearity range (mg.L^{-1})	R^2	LOD (ng.g^{-1})	LOQ (ng.g^{-1})
L2	0.005 - 2.50	0.998	0.17	0.57
D3		0.999	0.23	0.76
L3		0.999	0.50	1.67
D4		0.998	0.38	1.25
L4		0.998	1.43	4.76
D5		0.999	0.86	2.86
L5		0.998	3.75	12.50
D6		0.996	1.20	4.00

According to Table 9, it is possible to conclude that all the analysed compounds showed a linear behaviour. Normally, quality control laboratories admit three criteria to consider a calibration curve suitable (Harris, 2003): relative standard deviation of the slope (ratio between the standard deviation of the slope (s_a) and the slope (a)) has to be less than 5% ($s_a/a \times 100 < 5\%$); intercept should contain the origin ($b-s_b < 0 < b+s_b$), in order to guarantee a null response for a null concentration; the correlation factor (R) has to be higher than 0.995. The first criterion sometimes is no easy to obtain once the range of concentrations is lower, so the instrumental errors are more significant. These parameters were determined and are presented in Appendix 2. All calibration curves have relative standard deviation of the slope (s_a/a) below 5% and a correlation coefficient superior to 0.995. However not all calibration curves comply with the parameter that states that the intercept should contain the origin. This parameter was only observed for L4 and for L5, as it is possible to observe in Appendix 2 (Table A2).

The LODs of the studied compounds ranged between 0.17 and 3.75 ng.g^{-1}, and the LOQ ranged between 0.57 and 12.50 ng.g^{-1}. The highest values were obtained for L5 and the lowest for L2. Comparing these values

with those found in literature for the determination of siloxanes in cosmetics and personal care products it is possible to conclude that the values obtained in this study are generally lower than the ones already published. Only Lu et al. (2011), who applied SPE, obtained values in the same order of magnitude. However, the LOD and LOQ of L2 were much lower than those obtained by any other extraction method found in literature. So this means that, the developed methodology (QuEChERS-GC-MS), that was never used in the same context, allows distinguishing a lowest quantity of a substance.

Precision

The precision can be defined as the level of proximity between results for the same sample. To study the precision of the method the repeatability (intra-day precision) was determined. This was evaluated by the relative standard deviation (%RSD) of three replicates at different levels of spike (0.10 $mg.L^{-1}$, 0.50 $mg.L^{-1}$ and 1.00 $mg.L^{-1}$). The relative standard deviations are presented in Table 10 for each matrix studied.

Table 10: Precision (%RSD) for all the compounds analysed at three different spiked levels.

Type of product	Spike level ($mg.L^{-1}$)	%RSD							
		L2	D3	L3	D4	L4	D5	L5	D6
Moisturizer	0.10	7	6	2	3	1	6	3	8
	0.50	3	4	5	5	4	5	1	2
	1.00	2	4	7	6	3	4	2	1
Shower gel and gel toilet soap	0.10	1	5	2	6	4	2	6	6
	0.50	5	6	5	5	6	3	1	2
	1.00	1	4	3	1	1	2	1	2
Deodorant	0.10	1	3	5	6	1	1	2	6
	0.50	10	2	2	3	2	3	2	3
	1.00	8	4	2	1	1	1	2	6
Toothpaste	0.10	7	5	7	4	3	7	5	5
	0.50	41	15	3	2	3	2	7	4
	1.00	14	5	7	4	3	3	6	5
Solid toilet soap	0.10	13	2	7	2	10	5	2	2
	0.50	1	18	9	4	5	7	7	8
	1.00	12	3	5	9	1	4	5	3
Shampoo	0.10	1	5	5	4	1	5	1	3
	0.50	2	4	4	2	5	4	5	3
	1.00	2	2	1	4	7	1	1	4
Conditioner	0.10	4	4	3	4	4	3	1	3
	0.50	3	5	8	4	5	1	8	9
	1.00	4	1	5	1	4	7	3	7
Aftershave	0.10	1	7	4	7	4	7	4	12
	0.50	12	3	9	6	4	5	5	1
	1.00	12	11	4	3	2	1	3	3
Shaving foam/gel	0.10	5	1	3	2	3	9	1	8
	0.50	8	8	8	4	5	2	4	8
	1.00	3	6	5	3	3	1	5	6

Typically, values up to 10%, are considered acceptable taking into account the method employed and the range of working concentrations. The results, therefore, indicate that the method is precise once the values obtained are mostly below 10% (average of 5%). The highest RSD values were obtained for L2, ranging from 1 to 41% (average of 7%). This behaviour may be explained by the higher volatility displayed by this compound, which may lead to greater losses during the extraction processes and therefore, to higher relative

standard deviations. Higher RSD values were verified for toothpastes (average of 7%) and the lowest values of RSD for shampoo, shower gel/gel toilet soap and deodorant (average of 3%). Typically, samples spiked with higher levels of siloxanes led to lower precision values, which was observed in general. The relative standard deviation (RSD) showed a satisfactory precision for the applied methodology for all compounds analysed.

Accuracy

The accuracy measures the degree of proximity between the obtained and the expected result. This can be evaluated using a certified reference material or by recovery tests. In this case, the recovery tests were performed using spiked samples at three different levels (0.10 $mg.L^{-1}$, 0.50 $mg.L^{-1}$ and 1.00 $mg.L^{-1}$). In Table 11 is represented the mean recovery (%REC) from three replicates at each level.

Table 11: Recovery of siloxanes at different spiked levels.

Type of product	Spike level ($mg.L^{-1}$)	%REC							
		L2	D3	L3	D4	L4	D5	L5	D6
Moisturizer	0.10	26	43	74	83	102	87	104	90
	0.50	26	58	80	96	102	96	103	94
	1.00	30	65	84	97	104	95	103	96
Shower gel and gel toilet soap	0.10	42	104	75	90	102	103	94	98
	0.50	37	106	72	83	88	95	102	106
	1.00	53	103	79	90	95	100	100	100
Deodorant	0.10	22	61	62	40	86	76	107	95
	0.50	48	68	75	66	89	81	88	96
	1.00	44	73	82	78	95	90	95	97
Toothpaste	0.10	48	95	69	71	84	72	106	88
	0.50	39	62	75	76	94	90	99	98
	1.00	50	67	87	88	103	95	98	95
Solid toilet soap	0.10	29	75	68	87	89	101	77	78
	0.50	74	85	63	73	63	65	86	53
	1.00	38	105	37	67	38	60	73	87
Shampoo	0.10	108	84	104	92	96	94	105	92
	0.50	106	102	114	109	93	106	108	98
	1.00	99	105	107	105	84	107	110	94
Conditioner	0.10	109	104	106	101	98	98	91	102
	0.50	104	102	116	102	100	101	101	100
	1.00	99	104	105	102	98	88	104	95
Aftershave	0.10	23	31	55	92	74	86	94	95
	0.50	23	18	37	59	73	81	92	106
	1.00	52	61	62	79	82	83	82	99
Shaving foam/gel	0.10	79	62	93	98	97	91	102	94
	0.50	75	73	99	93	92	100	97	95
	1.00	71	76	100	90	94	98	95	85

The average recovery obtained for these tests was 84%, which is acceptable for this type of analysis. Lower recoveries were achieved for L2, which may be also explained for its high volatility. No significant relationship between the type of matrix and the recoveries values was found, but it seems that solid toilet soap and aftershave conducts to lower recoveries (around 68%). It is also possible to observe that with the increase of the spiked concentration levels, higher values of recoveries were reached in almost all cases.

Global uncertainty

According to the International Vocabulary of Basic and General Terms in Metrology, the uncertainty may be related with the result of a measurement, which characterizes the dispersion of values that could reasonably be attributed to the measured variable (ISO, 2006). Uncertainty may come from various sources, including the sampling, matrix effects and interferents, environmental conditions, measuring equipment, reference values, approximations and assumptions made in the method and random variations (Ellison et al., 2012).

There are several approaches for the calculation of uncertainties in a chemical analysis. The bottom-up methodology, the mostly used one, was proposed by the International Organization for Standardization (ISO) and by EUROCHEM/CITAC Guide (Ellison et al., 2000). The global uncertainty is determined by identifying, estimating and combining all sources of uncertainty associated with the result (Ratola et al., 2006). This methodology has the advantage to allow the interpretation and evaluation of individual contributions from the uncertainty sources, enabling the detection of the most significant (Tanase et al., 2015). In the present work, the approximation for estimating the uncertainty were applied and compared through the method proposed by EURACHEM (bottom-up). As mentioned above, the assessment of global uncertainty, based on the methodology described by EURACHEM (Ellison et al., 2000), takes into account the contributions of all sources of uncertainty. Four main sources of uncertainty were considered: the uncertainty associated to the standard preparation (U_1), calibration curve (U_2), precision (U_3) and the uncertainty associated to the accuracy (U_4). Accounting for the influence of each source, a global uncertainty (U_{global}) was calculated. The calculation procedure, including all expressions necessary to estimate the global uncertainty, and the results of the global uncertainty for each compound are presented in Appendix 3. In Table 12, the variation of the global uncertainty for the maximum concentration level (2.50 $mg.L^{-1}$) for each compound is shown.

Table 12: Limit values of global uncertainty for each compound.

Type of product	%U at maximum concentration							
	L2	D3	L3	D4	L4	D5	L5	D6
Moisturizer	3	4	6	5	3	4	3	3
Shower gel and gel toilet soap	9	4	3	2	2	3	2	4
Deodorant	7	4	3	2	2	2	3	5
Toothpaste	11	4	6	4	3	3	5	5
Solid toilet soap	10	3	4	6	2	3	4	4
Shampoo	3	2	2	4	5	2	2	5
Conditioner	4	2	4	2	4	5	3	5
Aftershave	10	8	4	3	2	2	3	4
Shaving foam/gel	3	5	4	3	3	2	4	6

A constant uncertainty was achieved for the upper and intermediate levels of the calibration range (Table 12). Though, when concentrations decrease, approaching the limits of detection, the global uncertainty rises exponentially. For the same compound, the global uncertainty is not significantly different varying the product type. Figure 8 presents the variation of the relative weight of each individual source of uncertainty for moisturizers, in this case for D5, the compound referred in the literature as the most used in toiletries. This compound may also be considered representative of the behaviour of the remaining compounds.

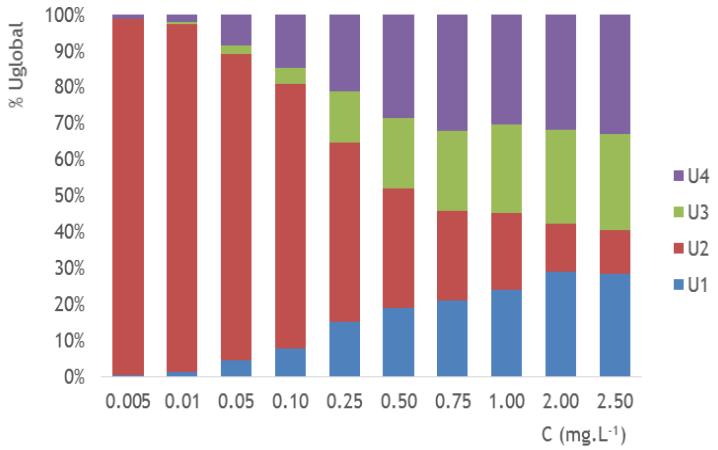


Figure 8: Variation of the relative weight of each individual source of uncertainty of moisturizers for D5.

As it can be seen (Figure 8 and Appendix 3), the relative contribution of the uncertainty of standard preparation (U_1) decreases when the concentration decreases too. Clearly, the importance of the calibration curve uncertainty (U_2) increases as it reaches towards the lower concentrations. In fact for the concentrations between 0.005 and 0.05 $mg.L^{-1}$, U_2 accounts for more than 80% of the global uncertainty. The contribution of the uncertainty related to the precision (U_3) decreases as the lower concentrations are reached. Regarding the accuracy (U_4), it has an important relative contribution for the global uncertainty at the highest concentrations (in some cases more than 50%), decreasing as it reaches the lowest ones.

5.2 Concentrations of volatile methylsiloxanes in cosmetics and personal care products

In order to estimate the pattern of use personal care products by the population of the Oporto region, the best selling brands of this kind of products were selected. It was also decided to study some products that are offered in hotels in the same region for comparison.

Volatile methylsiloxanes (VMSs) were detected in 131 of the 136 analysed products (96% of the samples), in concentrations ranging from 0.003 $\mu g.g^{-1}$ to 1,203.28 $\mu g.g^{-1}$ (Table 13). cVMSs were more frequently detected (94% of the samples) and at higher concentrations, reaching a maximum level of 1,203.28 $\mu g.g^{-1}$ for D3 in an adult shampoo. Linear VMSs were detected less often (54% of the samples) and in lower concentrations (maximum concentration of 8.61 $\mu g.g^{-1}$ for L3 in a hotel shampoo). Among the cVMSs, D4 and D6 were the most frequently detected compounds (87 and 80%, respectively), while L2 was the most detected linear VMS (35%). Higher concentrations were found for D3 (nd - 1,203.28 $\mu g.g^{-1}$; mean: 67.86 $\mu g.g^{-1}$) and D5 (nd - 753.53 $\mu g.g^{-1}$; mean: 39.82 $\mu g.g^{-1}$), followed by D6 (nd - 594.24 $\mu g.g^{-1}$; mean: 29.34 $\mu g.g^{-1}$) and D4 (nd - 267.03 $\mu g.g^{-1}$; nd: 20.80 $\mu g.g^{-1}$). L5 was the linear compound found in higher concentration (nd - 7.85 $\mu g.g^{-1}$; mean: 0.96 $\mu g.g^{-1}$).

The study of the linear siloxanes in this type of products is scarce, with only two papers available in the literature (Horii and Kannan, 2008; Lu et al., 2011). Horii and Kannan (2008) investigated 48 toiletries and cosmetic products marketed in USA and Japan, while Lu et al. (2011) studied 158 collected from China. Both studies are in line with the obtained results regarding the predominance of the two target classes, i.e. linear

siloxanes were detected less frequently than cyclic. Although this occurred in both studies, Lu et al. (2011) verified a detection frequency substantially closer between both classes (around 72% for cyclic and 70% for linear). Among linear VMSs, both studies verified that high molecular weight VMSs were more predominant and their concentrations were relatively higher in most products than those of low molecular weight VMSs. This tendency cannot be confirmed, since in the present work only the linear VMSs of low molecular weight were investigated. Analysing the cVMSs, the results obtained in this study are slightly different from those reported in literature. For instance, Horii and Kannan (2008) verified that the highest frequency of occurrence was for D5 (54%; <0.39 - 81,800 $\mu g.g^{-1}$), followed by D4 (50%; <0.35 - 272 $\mu g.g^{-1}$) and D6 (42%; <0.33 - 43,100 $\mu g.g^{-1}$). Wang et al. (2009) studied 252 products from Canada and also verified that D5 was the most frequently detected cVMS (14%; 20 - 683,000 $\mu g.g^{-1}$), followed by D6 (9%; 10 - 98,000 $\mu g.g^{-1}$) and D4 (5%; 10 - 11,000 $\mu g.g^{-1}$). Lu et al. (2011) reported that the highest concentrations and detection frequencies were found for the same compounds (64% for both cVMSs; D5: <0.005 - 1,110 $\mu g.g^{-1}$; D6: <0.022 - 367 $\mu g.g^{-1}$). Dudzina et al. (2014) also found a similar behaviour in a study of 51 personal care products marketed in Netherlands and Switzerland. They also concluded that D5 was present in higher concentrations (<0.72 - 356,000 $\mu g.g^{-1}$) and was predominant in almost all the samples. Evaluating the overall rate of occurrence of VMSs, Dudzina et al. (2014) and Lu et al. (2011) obtained similar results to those found in this study, i.e. VMSs were detected in almost all of the analysed samples

Analysing the best selling products, aftershaves contain higher average concentrations of linear VMSs (Σ L2-L5 = 2.01 $\mu g.g^{-1}$), being L5 the predominant. Higher mean concentrations of cVMSs were identified in facial cream (Σ D3-D6 = 150.68 $\mu g.g^{-1}$), shampoo (Σ D3-D6 = 117.26 $\mu g.g^{-1}$) and moisturizer (Σ D3-D6 = 95.65 $\mu g.g^{-1}$) for adults. D5 and D3 were the compounds detected in higher levels in those matrices. Mean concentrations of total VMSs were relatively high in facial creams (150.68 $\mu g.g^{-1}$), moisturizers (84.30 $\mu g.g^{-1}$), shampoos (58.90 $\mu g.g^{-1}$) and shower gels (28.01 $\mu g.g^{-1}$) for adults. In the first two types of products, D5 and D6 were detected in higher concentration levels (facial cream: 250.07 and 242.68 $\mu g.g^{-1}$, body moisturizer: 203.06 and 117.47 $\mu g.g^{-1}$, respectively). In the last two subcategories, D3 was the siloxane detected in higher amounts, with mean concentrations of 341.25 $\mu g.g^{-1}$ in shampoos and 86.98 $\mu g.g^{-1}$ in shower gels. The lowest mean concentrations were detected in toothpastes (0.02 $\mu g.g^{-1}$) (Table 13). A similar result was found by Horii and Kannan (2008), Lu et al. (2011) and Dudzina et al. (2014), who also concluded that toothpastes had the lowest levels. In the literature, there is no clear consensus on what are the products with the highest level of VMSs. Horii and Kannan (2008) concluded that average concentrations of total VMSs were high in hair care products. On the other hand, Wang et al. (2009) and Dudzina et al. (2014) found that deodorants contained the largest amounts (> 100,000 $\mu g.g^{-1}$), while Lu et al. (2011) concluded that it was the makeup products (417,000 $\mu g.g^{-1}$). Hotel amenities were also determined in the same conditions as the best selling products analysed. In this category, similar average concentrations of total VMSs were detected in shower gel (11.12 $\mu g.g^{-1}$), shampoo (13.36 $\mu g.g^{-1}$) and body moisturizer (15.05 $\mu g.g^{-1}$). L3 and D3 were the linear and cyclic siloxanes with the highest mean concentration (8.61 $\mu g.g^{-1}$ and 44.04 $\mu g.g^{-1}$ for shampoo, respectively). Comparing these values with those obtained for the best selling products of the same category, higher total average concentrations of VMSs were found in the latest products.

In this study, products intended to be only used for baby/children (body moisturizers, shower gel, shampoo and toothpaste) were also investigated. These PCPs presented lower levels of VMSs than products for adults (about ten times less), with the exception of children toothpastes. For body lotions, L5 was the only linear siloxane presented in concentrations above the limit of quantification (0.08 $\mu g.g^{-1}$) and cyclic siloxanes

D4 and D5 were detected with a higher mean concentration (0.07 $\mu g.g^{-1}$). In baby/children shower gel, only L3 was not detected, and the more prevalent were the cyclic D3 and D6 with 4.96 $\mu g.g^{-1}$ and 4.43 $\mu g.g^{-1}$, respectively. A similar situation was verified for baby/children shampoo, since D3 was detected in higher mean concentration (29.65 $\mu g.g^{-1}$). In children toothpastes, cVMSs had most significant concentrations, namely D6 and D3 with 0.38 $\mu g.g^{-1}$ and 0.31 $\mu g.g^{-1}$, respectively. To the authors' best knowledge, only one study available on literature provides information on the concentration levels of VMSs in personal care for babies/children (Wang et al., 2009), somewhat hindering a deeper discussion of the obtained results. In that study, 99 baby products marketed in Canada were analysed (oil, shampoo, lotion and diaper cream). Only cVMSs were investigated (D3-D6) and the authors concluded that their detection frequencies were low, when compared to the adult products (only detected in 3 samples). These compounds were detected in diaper and lotions, with concentration varying from 80 $\mu g.g^{-1}$ (D6) to 150 $mg.g^{-1}$ (D5). D5 and D6 were detected in higher concentration levels. As can be seen, in average, the detected levels mentioned in literature were higher than those obtained.

Concentration levels found in this study were similar to those reported by Chinese studies (Lu et al., 2011) and generally two to three orders of magnitude lower than those described in Japan and USA (Horii and Kannan, 2008), Canada (Wang et al., 2009) and also Switzerland and Netherlands (Dudzina et al., 2014). In fact, the consumption patterns differ geographically, which may explain some of these variations. Furthermore, some of these studies focused in the analysis of other type of PCPs, much more prone to have in their constitution a greater quantity of VMSs (e.g. stick or cream deodorants and makeup as liquid foundation). Another situation to consider is the growing number of scientific studies that have emerged in recent years related to human and environmental safety of these VMSs, as they are suspected to be potentially toxic (SCCP, 2005; Lassen et al., 2005). Thus, most manufacturing companies of toiletries have launched on the market "silicone free" products and it is possible that some old formulations have been adjusted, reducing the concentration of these compounds and replacing them by other solutions. In fact, the authors looked at the labels of studied products and found that most of them (around 80%) did not contain any VMS in their formulation list (either as pure substance or non-specifically as “dimethicone” or “cyclomethicone”) (Appendix 4). Therefore, in some cases, the presence of these compounds in very low concentrations may be explained by their use as raw materials for the production of other siloxane-based ingredients that are incorporated into personal care formulations or as impurities. According to the European Cosmetic legislation, when this happen these compounds are not recognised as ingredients, so they do not need to be present in the list of ingredients in the label of each product (European Parliament, 2009). Although the remaining 20% should contain mostly “dimethicone” (according to the information provided on the label), this study revealed a higher prevalence and concentration of cVMSs. Actually, these compounds (also known as “cyclomethicone”) are used as precursors in the production of polydimethylsiloxane (SCCP, 2005). Therefore, these polymers blends may contain some residual monomers, which are also regarded as impurities and, for that reason, are not indicated on the labels. Thus, it is possible that when “dimethicone” is used, residues of “cyclomethicone” may also be detected. As explained before, “dimethicone” is a mixture of fully methylated linear siloxane polymers with different chain sizes. Studies in the literature regarding the presence of these compounds in toiletries demonstrated that linear VMSs with a long chain (high molecular weight) are the most prevalent and also those found in higher concentration levels (Horii and Kannan, 2008; Lu et al. 2011). Since in this work was only studied the low molecular weight siloxanes (L2-L5), it is possible that the total concentration of linear VMSs may exceed the detected and also be higher than those found for the cyclic VMSs.

Table 13: Concentrations (μg.g^{-1}; mean, median and range) and frequency of detection (%) of siloxanes in cosmetics and PCPs from Oporto region.

Category	Product type		L2	L3	L4	L5	Σ L2-L5	D3	D4	D5	D6	Σ D3-D6	Total
Moisturizers	Adult body lotion/milk/ cream (n = 11)	Median	nd	<LOQ	0.23	0.08	0.14	3.32	8.95	119.59	3.50	5.59	3.86
		Mean	nd	<LOQ	0.23	0.29	0.28	4.06	22.98	203.06	117.47	95.65	84.30
		Range	nd	nd - <LOQ	nd - 0.23	nd - 0.98	nd - 0.98	nd - 10.76	nd - 105.13	nd - 753.53	0.11 - 471.18	nd - 753.53	nd - 753.53
		Frequency	0	18	36	45	73	73	82	91	100	100	100
	Hand creams (n = 3)	Median	0.16	0.04	nd	0.21	0.16	<LOQ	1.29	1.22	0.87	1.25	0.83
		Mean	0.16	0.04	nd	0.47	0.32	<LOQ	4.83	3.30	0.87	3.27	2.13
		Range	nd - 0.16	nd - 0.04	nd	0.11 - 1.10	nd - 1.10	nd - <LOQ	0.83 - 12.37	0.79 - 7.90	nd - 1.32	nd - 12.37	nd - 12.37
		Frequency	67	67	0	100	100	33	100	100	67	100	100
	Facial creams (n = 3)	Median	nd	<LOQ	<LOQ	nd	<LOQ	0.47	13.82	339.53	133.12	13.82	13.82
		Mean	nd	<LOQ	<LOQ	nd	<LOQ	0.47	13.82	250.07	242.68	150.68	150.68
		Range	nd	nd - <LOQ	nd - <LOQ	nd	nd - <LOQ	nd - 0.85	<LOQ - 23.72	3.06 - 407.62	0.68 - 594.24	nd - 594.24	nd - 594.24
		Frequency	0	33	33	0	33	67	100	100	100	100	100
	Baby and children body lotion/milk/ cream (n = 6)	Median	<LOQ	nd	nd	0.08	0.08	0.02	0.06	0.06	0.07	0.04	0.04
		Mean	<LOQ	nd	nd	0.08	0.08	0.02	0.07	0.07	0.06	0.05	0.05
		Range	<LOQ	nd	nd	nd - 0.08	nd - 0.08	nd - 0.02	0.03 - 0.14	nd - 0.15	nd - 0.08	nd - 0.15	nd - 0.15
		Frequency	100	0	0	17	100	83	100	67	83	100	100
Deodorants/ antiperspirants	Roll-on deodorants/ antiperspirants (n = 12)	Median	nd	nd	<LOQ	nd	<LOQ	1.52	2.21	0.80	0.74	1.30	1.30
		Mean	nd	nd	<LOQ	nd	<LOQ	8.86	2.87	1.34	0.74	4.21	4.21
		Range	nd	nd	nd - <LOQ	nd	nd - <LOQ	nd - 29.98	nd - 10.72	nd - 3.58	nd - 1.01	nd - 29.98	nd - 29.98
		Frequency	0	0	25	0	25	58	83	50	50	83	92

Table 13: Concentrations (μg.g^{-1}; mean, median and range) and frequency of detection (%) of siloxanes in cosmetics and PCPs from Oporto region (cont.).

Category	Product type		L2	L3	L4	L5	Σ L2-L5	D3	D4	D5	D6	Σ D3-D6	Total
Body and hair wash	Adult shower gel (n = 11)	Median	0.20	nd	nd	0.25	0.22	76.91	14.24	0.78	0.75	2.59	2.27
		Mean	0.20	nd	nd	0.25	0.22	86.98	25.67	3.73	1.86	29.56	28.01
		Range	nd - 0.20	nd	nd	nd - 0.25	nd - 0.25	nd - 309.59	nd - 93.14	nd - 15.74	nd - 6.50	nd - 309.59	nd - 309.59
		Frequency	9	0	0	9	18	82	82	91	91	100	100
	Baby and children shower gel (n = 9)	Median	0.08	nd	0.12	0.45	0.11	4.42	2.37	0.98	1.07	2.37	0.86
		Mean	0.08	nd	0.13	0.45	0.21	4.96	2.81	2.44	4.43	3.57	2.39
		Range	nd - 0.11	nd	nd - 0.16	nd - 0.80	nd - 0.80	nd - 8.01	nd - 5.34	nd - 7.78	Nd - 11.28	nd - 11.28	nd - 11.28
		Frequency	22	0	44	22	67	33	89	89	78	89	100
	Adult shampoo (n = 14)	Median	0.25	0.25	0.78	0.78	0.39	268.88	79.44	17.70	19.20	30.08	1.11
		Mean	0.28	0.25	0.84	0.80	0.54	341.25	91.45	18.36	17.98	117.26	58.90
		Range	0.12 - 0.80	0.15 - 0.39	0.40 - 1.34	0.39 - 1.28	0.12 - 1.34	28.89 - 1203.28	3.87 - 267.03	1.00 - 39.94	0.69 - 42.01	0.69 - 1203.28	0.12 - 1203.28
		Frequency	100	100	100	100	100	100	100	100	100	100	100
	Adult hair conditioner (n = 8)	Median	nd	0.15	0.63	0.55	0.39	19.96	22.47	19.18	23.69	21.53	14.30
		Mean	nd	0.15	0.64	0.54	0.45	19.60	34.03	19.35	28.46	25.74	18.52
		Range	nd	nd - 0.25	nd - 0.98	nd - 0.78	nd - 0.98	nd - 40.42	0.59 - 117.36	1.56 - 49.46	0.73 - 62.31	nd - 117.36	nd - 117.36
		Frequency	0	50	50	50	50	75	100	100	100	100	100
	Baby and children shampoo (n = 8)	Median	0.12	0.25	0.87	0.71	0.21	34.59	1.51	0.87	2.50	1.54	0.65
		Mean	0.12	0.24	0.69	0.59	0.38	29.65	4.75	2.37	2.50	9.09	4.73
		Range	nd - 0.18	nd - 0.41	nd - 1.00	nd - 0.87	nd - 1.00	nd - 43.79	nd - 20.13	nd - 7.39	nd - 4.41	nd - 43.79	nd - 43.79
		Frequency	50	50	38	38	50	38	63	50	25	75	75

Table 13: Concentrations (μg.g^{-1}; mean, median and range) and frequency of detection (%) of siloxanes in cosmetics and PCPs from Oporto region (cont.).

Category	Product type		L2	L3	L4	L5	Σ L2-L5	D3	D4	D5	D6	Σ D3-D6	Total
Toilet soaps	Solid soap (n = 9)	Median	<LOQ	nd	nd	nd	<LOQ	3.96	0.27	5.36	5.01	2.61	2.61
		Mean	<LOQ	nd	nd	nd	<LOQ	3.96	0.71	4.57	5.27	3.49	3.49
		Range	nd - <LOQ	nd	nd	nd	nd - <LOQ	nd - 4.39	0.09 - 2.43	0.10 - 9.16	nd - 11.06	nd - 11.06	nd - 11.06
		Frequency	22	0	0	0	22	22	100	100	89	100	100
	Gel soap (n = 6)	Median	<LOQ	nd	nd	0.13	0.13	12.03	1.18	0.05	0.20	0.57	0.54
		Mean	<LOQ	nd	nd	0.13	0.13	12.04	1.17	0.09	0.18	4.33	4.11
		Range	nd - <LOQ	nd	nd	nd - 0.13	nd - 0.13	0.54 - 22.01	nd - 1.72	nd - 0.27	nd - 0.30	nd - 22.01	nd - 22.01
		Frequency	50	0	0	17	67	50	67	67	83	100	100
Dentifrice products	Adult toothpaste (n = 6)	Median	<LOQ	nd	nd	nd	<LOQ	0.01	0.02	nd	0.02	0.01	0.01
		Mean	<LOQ	nd	nd	nd	<LOQ	0.01	0.02	nd	0.04	0.02	0.02
		Range	<LOQ	nd	nd	nd	nd - <LOQ	nd - 0.01	0.01 - 0.05	nd	nd - 0.09	nd - 0.09	nd - 0.09
		Frequency	100	0	0	0	100	67	100	0	83	100	100
	Children toothpaste (n = 6)	Median	<LOQ	nd	nd	nd	<LOQ	0.42	0.11	0.14	0.14	0.20	0.20
		Mean	<LOQ	nd	nd	nd	<LOQ	0.31	0.14	0.13	0.13	0.23	0.23
		Range	nd - <LOQ	nd	nd	nd	nd - <LOQ	nd - 0.59	0.02 - 0.30	nd - 0.27	nd - 0.27	nd - 0.59	nd - 0.59
		Frequency	50	0	0	0	50	83	100	83	83	100	100
Shaving products	Shaving foam/gel (n = 7)	Median	nd	nd	nd	nd	nd	1.68	<LOQ	3.15	2.12	2.06	2.06
		Mean	nd	nd	nd	nd	nd	1.76	<LOQ	3.15	2.12	2.11	2.11
		Range	nd	nd	nd	nd	nd	nd - 2.43	nd - <LOQ	nd - 3.15	nd - 3.96	nd - 3.96	nd - 3.96
		Frequency	0	0	0	0	0	43	14	14	29	57	71
	Aftershave (n = 4)	Median	0.005	0.78	1.23	4.02	0.78	1.27	1.08	106.03	1.76	1.67	1.38
		Mean	0.005	0.78	1.23	4.02	2.01	1.57	2.60	106.03	34.14	25.47	18.96
		Range	nd - 0.005	nd - 0.78	nd - 1.23	nd - 7.85	nd - 7.85	0.19 - 3.55	0.14 - 8.11	nd - 209.60	nd - 100.28	nd - 209.60	nd - 209.60
		Frequency	25	25	25	50	50	100	100	50	75	100	100

Table 13: Concentrations (μg.g^{-1}; mean, median and range) and frequency of detection (%) of siloxanes in cosmetics and PCPs from Oporto region (cont.).

Category	Product type		L2	L3	L4	L5	Σ L2-L5	D3	D4	D5	D6	Σ D3-D6	Total
Hotel amenities	Body lotion/milk/ cream (n = 6)	Median	5.69	0.17	<LOQ	nd	2.93	10.72	3.98	1.53	1.10	2.58	2.58
		Mean	5.69	0.17	<LOQ	nd	2.93	10.28	3.82	42.61	7.53	16.06	15.05
		Range	nd - 5.69	nd - 0.17	nd - <LOQ	nd	nd - 5.69	4.68 - 14.25	1.74 - 5.50	0.27 - 248.56	0.27 - 40.91	0.27 - 248.56	nd - 248.56
		Frequency	17	17	17	0	17	100	100	100	100	100	100
	Shower gel (n = 3)	Median	0.07	nd	1.76	5.71	0.94	6.13	4.12	18.89	1.29	5.16	4.12
		Mean	0.07	nd	1.76	5.71	1.90	14.50	19.97	18.89	6.00	14.47	11.12
		Range	nd - 0.12	nd	nd - 1.76	nd - 5.71	nd - 5.71	5.16 - 32.20	0.69 - 55.09	nd - 36.96	0.03 - 16.69	nd - 55.09	nd - 55.09
		Frequency	67	0	33	33	100	100	100	67	100	100	100
	Shampoo (n = 4)	Median	2.43	8.61	2.44	2.70	2.57	23.74	10.01	0.73	0.45	3.92	2.96
		Mean	2.43	8.61	2.44	2.70	4.05	44.04	14.48	2.77	1.46	15.69	13.36
		Range	nd - 2.43	nd - 8.61	nd - 2.44	nd - 2.70	nd - 8.61	5.94 - 122.74	1.84 - 36.07	0.32 - 9.30	0.33 - 4.61	0.32 - 122.74	nd - 122.74
		Frequency	25	25	25	25	25	100	100	100	100	100	100
All products (n = 136)		Median	0.18	0.24	0.76	0.68	0.30	7.07	2.37	2.48	1.43	2.77	1.22
		Mean	0.49	0.57	0.80	0.96	0.73	67.86	20.80	39.82	29.34	38.69	29.85
		Range	nd - 5.69	nd - 8.61	nd - 2.44	nd - 7.85	nd - 8.61	nd - 1203.28	nd - 267.03	nd - 753.53	nd - 594.24	nd - 1203.28	nd - 1203.28
		Frequency	35	22	27	28	54	70	87	76	80	94	96

5.3 Additional implications for consumer exposure assessment

Dermal sorption and inhalation are considered the main exposure routes for siloxanes since they are present in PCPs, which are directly used by consumers (Lu et al., 2011). Human dermal adsorption has been poorly studied along the years (Reddy et al., 2007; Jovanovic et al., 2008). Reddy et al. (2007) performed *in vitro* tests with human volunteers exposed through the axilla region, which is considered to be relatively permeable and normally exposed to antiperspirants, one of the matrices with the highest expected levels of D4 and D5. In this study, only 0.12% and 0.30% of D4 absorption was predicted to men and women, respectively. For D5, 0.05% of the applied dose was absorbed. Low dermal absorption of D4 and D5 was also reported by Jovanovic et al. (2008). Around 0.50% and 0.04% of absorption was reached, respectively, in human skin. Both studies considered the loss of volatile D4 and D5 from skin, either straight from the skin surface, or indirectly through back diffusion towards the skin surface, the main reason for low absorption of these siloxanes. Jovanovic et al. (2008) also evaluated the *in vitro* human dermal absorption of cyclic siloxane D6. Once more, extremely low levels of absorption were found (near 0%) (Johnson et al., 2012). As can be seen, in all these studies the percutaneous absorption seems to decrease with increasing molecular weight and lipophilicity of the cyclic siloxanes. Wang et al. (2009) studied the volatilization potential of D5 from the skin, after the application of a roll-on antiperspirant. They verified that 60% of the applied amount of D5 remained on the skin for up to 6 hours after use, showing that evaporative loss of D5 is not the principal reason for its low absorption rate. The same authors claim that the evaporative loss may be greater for D4, since it presents a greater volatile nature. *In vitro* human dermal absorption studies with "dimethicone" were also performed (Teasdale et al., 2015). Absorption of 0.2% and 0.1% of the applied dose of polydimethylsiloxane 10 cST and 350 cST was determined. Therefore, based on these existing studies, an average value of 1% was estimated by the authors.

There are different approaches that can be used to determine dermal exposure. The methods range from employing very simple deterministic exposure models to utilizing more sophisticated probabilistic frameworks (Dudzina et al., 2014). In the present work, the daily dermal exposure to eight different siloxanes (L2 to L5 and D3 to D6) through the application of twelve most commonly used types of toiletries (Table 14), was estimated using the Nakata dermal exposure model (Nakata et al., 2015). For the evaluation of the daily dermal exposure some parameters were taking into account, as product type (e.g. rinse-off and leave-on), the amount of product used per application, its frequency of application, the target group of use (adults or baby/children) and the retention factor of the product, which were established based on data provided from other European Union surveys (Biesterbos et al., 2013; Bremmer et al., 2006; SCCS, 2012). To give a general overview, two different scenarios were considered for this estimation: 1) the average concentrations of VMSs were used, 2) the worst-case scenario that consists in using the highest concentration values of VMSs. The daily dermal exposure was calculated according to the Eq. 1 (Nakata et al., 2015):

$$D_{exp} = \sum_{i=1}^{n} \sum_{j=1}^{m} \frac{C_j \times A_i \times F_i \times R_i}{BW} \quad (1)$$

where D_{exp} represents the daily dermal exposure *per capita* (μg.kg$_{bw}^{-1}$.day^{-1}), i the type of toiletry product, n the number of personal care products, j the type of siloxane (e.g. L2, L3, D3, etc.), m the number of siloxanes, C the siloxane concentration in the toiletry product used (μg.g^{-1}), A represents the amount of product applied per application (g.event^{-1}), F represents the frequency of application (events.day^{-1}), R represents the retention factor (dimensionless), and BW represents the average body weight (kg). The retention factor

denotes the product amount that may be retained on the skin and should vary between 0 (entire product is rinsed-off) and 1 (none of the product is rinsed-off). The daily dermal uptake was also calculated multiplying the daily dermal exposure by permeation factors, which indicate the permeation level of the target compounds through the skin.

Table 14 presents adult daily dermal exposure to siloxanes through the application of PCPs. The mean and maximum daily dermal exposure for adults to siloxanes would be 25.04 and 89.25 $\mu g.kg_{bw}^{-1}.day^{-1}$, respectively. Among the considered product subcategories, the main contributors for adult dermal exposure were body moisturizers (mean: 20.71 $\mu g.kg_{bw}^{-1}.day^{-1}$, maximum: 79.84 $\mu g.kg_{bw}^{-1}.day^{-1}$), followed by facial creams (mean: 2.98 $\mu g.kg_{bw}^{-1}.day^{-1}$; maximum: 6.02 $\mu g.kg_{bw}^{-1}.day^{-1}$) and aftershaves (mean: 0.92 $\mu g.kg_{bw}^{-1}.day^{-1}$; maximum: 2.03 $\mu g.kg_{bw}^{-1}.day^{-1}$). The exposure to siloxanes through the remaining toiletry products is considered less significant. More detailed information can be found in Appendix 5. In fact, the exposure to body moisturizers represents 83% of the mean daily dermal exposure, with higher values for D5 (12.08 $\mu g.kg_{bw}^{-1}.day^{-1}$) and D6 (6.99 $\mu g.kg_{bw}^{-1}.day^{-1}$). In relation to the maximum values of daily dermal exposure, the same body moisturizers reached values of 89% of the exposure. It is also important to notice that cyclic siloxanes are the most significant for the human daily dermal exposure (specially D5 and D6), since they have been detected at higher levels.

As mentioned before, there is little information available on the dermal permeation factors of siloxanes. Therefore, taking into account the uncertainties regarding the dermal penetration rates of these chemicals and of course, their variability with the vehicle tested, an average permeation factor of 1% was considered in this work. Assuming this value, an average dermal uptake of 0.25 $\mu g.kg_{bw}^{-1}.day^{-1}$ was achieved.

A similar study was performed for children/baby products to estimate their daily dermal exposure. Due to their state of development, baby and children present a thinner and less resistant skin, making them a more vulnerable group (Paller et al., 2011). According with this, the estimated daily dermal exposure to siloxanes in children is presented in Table 15.

The mean and maximum daily dermal exposure for children/baby to siloxanes would be 0.35 and 0.65 $\mu g.kg_{bw}^{-1}.day^{-1}$, respectively. Also for this target group, body moisturizer was the product type that had the highest mean and maximum daily dermal exposure, 0.12 and 0.19 $\mu g.kg_{bw}^{-1}.day^{-1}$, respectively. This was followed by shower gel, with a mean daily dermal exposure of 0.09 $\mu g.kg_{bw}^{-1}.day^{-1}$ and a maximum of 0.20 $\mu g.kg_{bw}^{-1}.day^{-1}$ and shampoo with 0.08 and 0.16 $\mu g.kg_{bw}^{-1}.day^{-1}$, respectively. In fact, body moisturizers represent around 34% of the mean daily dermal exposure, while shower gel and shampoo 26 and 24%, respectively. Details about the mean and maximum values of the daily dermal exposure for each compound and product are presented in Appendix 5. It is possible to observe that D3 is the compound with highest total value of mean daily dermal exposure (about 0.12 $\mu g.kg_{bw}^{-1}.day^{-1}$), thus accounting for 34% of the aggregate exposure. A maximum of 0.19 $\mu g.kg_{bw}^{-1}.day^{-1}$ (29%) is also expected. On the other hand, solid soaps contribute with less than 1% for the total dermal exposure (mean: 0.0008 $\mu g.kg_{bw}^{-1}.day^{-1}$). Once more, the cyclic siloxanes are the dominant compounds (around 90%) in the daily dermal exposure. To determine the daily dermal uptake, the same value of 1% was assumed due to the lack of information. Therefore, an average dermal uptake of 0.35 $\mu g.kg_{bw}^{-1}.day^{-1}$ is expected.

Since adult and children/baby products were analysed, the results from these two groups were compared in order to study the influence on the average daily dermal exposure. Considering the same product types, the baby/children dermal exposure to the totality of siloxanes (L2-D6) is about sixty times lower than

Table 14: Estimated adult daily dermal exposure to siloxanes through toiletries.

Category	Product type	Amount per application ($g.event^{-1}$)	Frequency of application ($events.day^{-1}$)	Retention factor	Total concentration of siloxanes ($\mu g.g^{-1}$)		Daily dermal exposure ($\mu g.kg_{bw}^{-1}.day^{-1}$)		Daily dermal uptake ($\mu g.kg_{bw}^{-1}.day^{-1}$)	
					Mean	Maximum	Mean	Maximum	Mean	Maximum
Moisturizers	Body lotion/milk/cream	8.50	0.42	1.00	348.09	1341.81	20.71	79.84	0.21	0.80
	Hand cream	0.50	0.80	1.00	9.67	22.90	0.06	0.15	0.001	0.002
	Facial cream	0.40	0.88	1.00	507.03	1026.43	2.97	6.02	0.03	0.06
Toilet soaps	Solid soap	0.80	0.50	0.01	14.51	27.03	0.001	0.002	0.00	0.00
	Gel soap	1.00	4.50	0.01	13.62	24.43	0.01	0.02	0.0001	0.0002
Body and hair wash	Shower gel	6.30	0.71	0.01	118.68	425.40	0.09	0.32	0.001	0.003
	Shampoo	4.80	0.50	0.01	471.20	1556.07	0.19	0.62	0.002	0.01
	Hair conditioner	4.90	0.43	0.01	102.78	271.56	0.04	0.10	0.0004	0.001
Dentifrice products	Toothpaste	1.10	2.00	0.05	0.07	0.15	0.0001	0.0003	0.00	0.00
Deodorants	Roll-on deodorant	0.20	1.00	1.00	13.81	45.29	0.05	0.15	0.005	0.002
Shaving products	Shaving foam/gel	3.55	0.43	0.01	7.03	9.54	0.001	0.002	0.00	0.00
	Aftershave	0.80	0.46	1.00	150.37	331.41	0.92	2.03	0.01	0.02
						TOTAL ($\mu g.kg_{bw}^{-1}.day^{-1}$)	**25.04**	**89.25**	**0.25**	**0.89**

Adult body weight: 60 kg

Table 15: Estimated daily dermal exposure to siloxanes contained in selected toiletries in children.

Category	Product type	Amount per application ($g.event^{-1}$)	Frequency of application ($events.day^{-1}$)	Retention factor	Total concentration of siloxanes ($\mu g.g^{-1}$)		Daily dermal exposure ($\mu g.kg_{bw}^{-1}.day^{-1}$)		Daily dermal uptake ($\mu g.kg_{bw}^{-1}.day^{-1}$)	
					Mean	Maximum	Mean	Maximum	Mean	Maximum
Moisturizers	Body lotion/milk/cream	4.53	2.00	1.00	0.29	0.46	0.12	0.19	0.0012	0.0019
Toilet soaps	Solid soap	0.25	0.50	0.01	14.51	27.03	0.001	0.002	0.00001	0.00002
	Gel soap	0.29	4.50	0.01	13.62	24.43	0.01	0.02	0.00008	0.0002
Body and hair wash	Shower gel	10.29	1.23	0.01	15.29	33.48	0.09	0.20	0.0009	0.0020
	Shampoo	7.30	0.60	0.01	40.91	78.17	0.08	0.16	0.0008	0.0016
Dentifrice products	Toothpaste	0.53	2.00	1.00	0.96	1.72	0.05	0.08	0.0005	0.0008
						TOTAL ($\mu g.kg_{bw}^{-1}.day^{-1}$)	**0.35**	**0.65**	**0.003**	**0.006**

Body weight: 21.7 Kg

adults' exposure. The results also indicated that the exposure to body moisturizers is dominant for adults (99%), but also for baby/children (34%). Shampoo and shower gel are also important to baby/children dermal exposure (around 25%).

There are other studies that estimated the exposure profiles to cyclic and linear siloxanes from PCPs, such as the one performed by Horii and Kannan (2008). In this study, D5 was the cyclic siloxane with a higher dermal exposure rate (about 233,000 $\mu g.day^{-1}$ or 3,883 $\mu g.kg_{bw}^{-1}.day^{-1}$), reaching the highest values for hair conditioners (162,000 $\mu g.day^{-1}$ or 2,700 $\mu g.kg_{bw}^{-1}.day^{-1}$). Regarding the linear siloxanes (sum of L4 to L14), facial creams seem to be the products that contribute with higher exposure rate (49,900 $\mu g.day^{-1}$ or 832 $\mu g.kg_{bw}^{-1}.day^{-1}$). These values are very high when compared to those obtained in this study. Also Lu et al. (2011) estimated the exposure profiles of siloxanes through dermal application of personal care products. Trace levels of siloxanes in toothpastes suggest that this product is a minor source of exposure, which is similar to what happens in the present study, as toothpaste present an exposure rate of 0.0003 $\mu g.kg_{bw}^{-1}.day^{-1}$. Moreover, the total daily exposure rate to siloxanes presented by Lu et al. (2011) from the use of PCP was estimated in 4,510 $\mu g.day^{-1}$ (around 75 $\mu g.kg_{bw}^{-1}.day^{-1}$) in China. This value is similar to the one obtained in the present study. The highest contributor to the exposure quantities was liquid foundation with 1,250 $\mu g.day^{-1}$ (21 $\mu g.kg_{bw}^{-1}.day^{-1}$), followed by hair conditioner with 750 $\mu g.day^{-1}$ (12.5 $\mu g.kg_{bw}^{-1}.day^{-1}$). Wang et al. (2009) also try to predict the daily dermal exposure to D4 and D5, through the application of a body lotion or antiperspirant. They verified that for D4 a daily dermal exposure of 500 $\mu g.day^{-1}$ (8.3 $\mu g.kg_{bw}^{-1}.day^{-1}$) and 10 $\mu g.day^{-1}$ (0.17 $\mu g.kg_{bw}^{-1}.day^{-1}$) is expected by the use of body lotions and antiperspirants, respectively. Regarding D5, higher values are expected (100 $\mu g.day^{-1}$ in body lotion and 200 $\mu g.day^{-1}$ in antiperspirant). In general, these values are higher than those obtained in this study. Dudzina et al. (2014) estimated the daily exposure to D4 and D5 through application of most usually PCPs subcategories used, as body lotion, facial cream, hand cream, deodorant, liquid foundation and hair conditioner. Here, daily external exposure to D5 was estimated in higher amounts (maximum of 1,224,000 $\mu g.day^{-1}$, i.e. 20,400 $\mu g.kg_{bw}^{-1}.day^{-1}$) than D4 (10,800 $\mu g.day^{-1}$, i.e. 180 $\mu g.kg_{bw}^{-1}.day^{-1}$). For D5, body lotions with 330,000 $\mu g.day^{-1}$ (5,500 $\mu g.kg_{bw}^{-1}.day^{-1}$) and liquid foundation with 221,000 $\mu g.day^{-1}$ (3,683 $\mu g.kg_{bw}^{-1}.day^{-1}$) proved to be the highest contributors to the dermal exposure. While for D4, deodorants/antiperspirants non-spray (7,600 $\mu g.day^{-1}$ or 127 $\mu g.kg_{bw}^{-1}.day^{-1}$) and body lotions (2,900 $\mu g.day^{-1}$ or 48 $\mu g.kg_{bw}^{-1}.day^{-1}$) were the most relevant classes of products. Generally, all the authors verify that D5 is the siloxane that most contributes to the dermal exposure, which is in line with the present study.

The results regarding the products that most contribute for the aggregate exposure are quite dispersed, clearly demonstrating that consumption patterns differ geographically. The differences obtained in the dermal exposure levels are, once more, related to the concentration values of siloxanes in the products analysed, which are lower than the values observed in the mentioned studies.

Overall, these preliminary results of aggregate consumer exposure indicate that the dermal application of these compounds does not seem to lead to increased health risks, since most dermal toxicity studies point out for significantly higher values. For instance, a No-Observed-Effect-Level (NOEL) of 960 $mg.kg_{bw}^{-1}.day^{-1}$ was found in a study performed with rabbits with dermal application (28 days) of D4. Similarly, a NOEL of 1600 $mg.kg_{bw}^{-1}.day^{-1}$ was found in studies conducted in rats with dermal application of D5 up to 4 weeks (SCCS, 2012).

5.4 Estimation of "down-the-drain" emissions

It as been sugested that the majority of volatile methylsiloxanes (namely, D5), will be lost from "leave-on" personal care products to the atmosphere (Egmond et al., 2013), where it is expected to be broken down via reaction with hydroxyl radicals. However, not all emissions will be released to the atmosphere. There are actually "rinse-off" products that are likely to be discharged into the sewage system reaching, consequently, the wastewaters (Egmond et al., 2013).

As mentioned before, benefits, such as ease of spreading and the capability to carry and release active ingredients are directly related to the physicochemical properties of siloxanes (e.g. low surface tension, hydrophobicity and volatility). Volatility and hydrophobicity are also key parameters that influence the environmental fate and behaviour of these compounds after use and in the emissions to the environment (Brooke et al., 2005). Thus, their use in either leave-on PCPs, such as skin creams, deodorants/antiperspirants, or rinse-off (e.g. liquid soaps, conditioners and shampoo), combined with these unique properties may greatly affect the total amounts being discharged "down-the-drain". As verified before, the inclusion levels of siloxanes in PCPs is quite variable and their dermal absorption potential seems to be relatively low (Reddy et al., 2007), making them prone to be washed-off. Therefore, in this study the emission "down-the-drain" *per capita* for linear and cyclic siloxanes was determined, according to the Eq. 2, adapted from Gouin et al. (2013).

$$E_m = \sum_{i=1}^{n}\sum_{j=1}^{m} C_j \times A_i \times F_i \times (1 - R_i) + \sum_{i=1}^{n}\sum_{j=1}^{m} C_j \times A_i \times F_i \times R_i \times (1 - F_{dermal}) \times \left(1 - F_{evap}\right) \quad (2)$$

where E_m represents the estimated "down-the-drain" emission *per capita*, F_{dermal} represents the rate of penetration of these chemicals through the skin, and F_{evap} represents the evaporation factor that reflects the volatilization potential from the skin surface in 24 hours. As previously mentioned, there is little information available on the rate of penetration of siloxanes through the skin. However, based on the existing studies, an average value of 1% was estimated by the authors. For the evaporation factor was assumed a value of 95% provided by Montemayor et al. (2013). The values for "down-the-drain" emission *per capita* were estimated for cyclic and linear siloxanes and are presented in Table 16. Details about the values of "down-the-drain" emission for each compound and product are presented in Appendix 6.

Table 16: Estimates of "down-the-drain" siloxanes emissions for diverse product types.

Category	Product type	Estimated *per capita* "down-the-drain" emissions of total siloxanes (µg.day^{-1})		
		Minimum	Mean	Maximum
Moisturizers	Body lotion/milk/cream	0.02	61.51	237.12
	Hand cream	0.03	0.19	0.45
	Facial cream	0.07	8.83	17.88
Toilet soaps	Solid soap	0.08	5.75	10.71
	Gel soap	2.39	60.69	108.89
Body and hair wash	Shower gel	26.06	525.82	5,944.87
	Shampoo	20.04	1,120.13	3,699.06
	Hair conditioner	6.00	214.49	566.73
Dentifrice products	Toothpaste	0.02	0.15	0.31
Deodorants	Roll-on deodorant	0.00	0.14	0.45
Shaving products	Shaving foam/gel	0.00	10.62	14.42
	Aftershave	0.01	2.74	6.04
	Total (µg.day^{-1})	54.71	2,011.05	10,606.93

Due to the significant loss of siloxanes by evaporation, the leave-on products do not contribute significantly to the mass loading of wastewater treatment systems, having a maximum total value of 255.90 $\mu g.day^{-1}$ (2%). However, the rinse-off products represent the greater emission route (98%). Thus, shampoo was the product that seems to contribute more to the siloxanes "down-the-drain" emission, with a mean value of 1,120.13 $\mu g.day^{-1}$, followed by shower gels with 525.82 $\mu g.day^{-1}$ and then hair conditioners with 214.49 $\mu g.day^{-1}$. Cyclic siloxanes, as predictable, showed the highest values for this estimation (with a highest total mean value of 1,296.05 $\mu g.day^{-1}$ for D3), since they are in higher amounts in the cosmetics and PCPs analysed and are, in general, less volatile than linear siloxanes. D5 is the siloxane with the highest estimated maximum *per capita* "down-the-drain" emission, with 3,690.50 $\mu g.day^{-1}$ (34.8%), with predominance in shower gels (3,338.49 $\mu g.day^{-1}$). Once, there are no studies that report an estimation of "down-the-drain" discharges of siloxanes, it is difficult to compare the obtained values with the ones published in literature. For this, it is possible to compare with the mass loadings estimated in some studies. As it was predictable, cyclic siloxanes are the siloxanes with higher mass loadings once they are predominant in wash-off products. As reported in the literature, D5 is the cyclic siloxane with highest values of mass loadings presenting variable values as 0.0027 $g.capita^{-1}.day^{-1}$, 0.0016 $g.capita^{-1}.day^{-1}$, 0.0005 $g.capita^{-1}.day^{-1}$, 0.0715 $mg.capita^{-1}.day^{-1}$, 0.0072 $g.capita^{-1}.day^{-1}$ in studies reported by Egmond et al. (2013), Xu et al. (2013), Bletsou et al. (2013), Wang et al. (2015a,b), respectively. Comparing the values, it is possible to conclude that the obtained values are lower than the ones described in the literature. This can be explained by the fact that these mass loadings correspond to the total of siloxanes found in influents, and the values present in this study only correspond to the discharge of siloxanes from cosmetics and personal care products.

6 Conclusions

The concentration levels of eight volatile methylsiloxanes (L2 to L5 and D3 to D6) in different adult and children/baby cosmetics and personal care products (moisturizers, toilet soaps, body and hair wash, dentifrice products, deodorants/antiperspirants and shaving products) were investigated in the best selling products in Oporto region (Portugal). To accomplish that task, a QuEChERS methodology coupled to GC-MS analysis were developed and validated.

In those analysed samples, volatile methyl siloxanes were detected in 96%, reaching a maximum concentration of 1,203.28 $\mu g.g^{-1}$ (D3 in adult shampoo). Cyclic siloxanes were detected more often (94%) and in higher concentration levels than linear compounds (only detected in 54% of the samples). From cyclic siloxanes, D4, D6 and D5 were the more frequently detected compounds (87, 80%, and 76%, respectively), while L2 was the compound most detected from the linear class (35%). Analysing the total concentrations, higher values were detected in facial cream (150.68 $\mu g.g^{-1}$), adult body moisturizers (84.30 $\mu g.g^{-1}$) and adult shampoo (58.90 $\mu g.g^{-1}$). Baby lotions (0.05 $\mu g.g^{-1}$) and toothpastes (0.13 $\mu g.g^{-1}$) contained the lowest concentration levels.

Dermal exposure was estimated based on the concentrations of siloxanes in the studied personal care products and the average daily usage amounts of those consumer products. The estimated adult mean daily dermal exposure to siloxanes was calculated as 25.04 $\mu g.kg_{bw}^{-1}.day^{-1}$ and children's exposure was 0.35 $\mu g.kg_{bw}^{-1}.day^{-1}$. Body moisturizer was the highest contributor to those exposure amounts. Comparing these values with some dermal toxicity data, it seems that the dermal exposure to these compounds does not seem to lead to health risks.

Finally, considering the studied aggregated consumption pattern of adult personal care products, the amount of siloxanes release "down-the-drain" into the sewage systems was estimated. It is expected, an average emission *per capita* of 2,011.05 $\mu g\ day^{-1}$. Analysing the maximum emissions *per capita*, D5 seems to be the more prevalent compound with 3,690.50 $\mu g\ day^{-1}$, followed by D3 (3,098.25 $\mu g\ day^{-1}$) and D6 (2,424.85 $\mu g\ day^{-1}$). Wash-off products, namely shower gel and shampoo, are the greater source of volatile methylsiloxanes in the sewage systems (97%).

The results of this thesis have been presented in four conferences/seminar and one paper has already been submitted to an international peer-reviewed journal (the different abstracts are presented in Appendix 7):

- D. Capela, L. Santos, V. Homem, *New analytical methodology based on QuEChERs followed by GC-MS to determine siloxanes in personal care products*, Encontro de Jovens Investigadores da Universidade do Porto - 8ª edição, 13-15 May 2015, Porto, Portugal (Oral presentation)

- D. Capela, Short-seminar *Siloxanes in cosmetics and personal care products*, FEUP, Product Chemistry and Technology, Master in Bioengineering and Chemical Engineering, 13 October 2015

- V. Homem, D. Capela, A. Alves, L. Santos, *Assessment of siloxanes release into the environment by personal care products*, European Meeting on Environmental Chemistry (EMEC 16), 30 November-3 December 2015, Torino, Italy (Oral presentation)

- N. Ratola, V. Homem, D. Capela, S. Ramos, J.A. Silva, C. Cunha, E. Silva, I. Magalhães, R. Araújo, L. Santos, A. Alves, *QuEChERS of micropollutants: mission in several matrices*, XVI COLACRO/9º Encontro Nacional de Cromatografia, 5-9 January 2016, Lisbon, Portugal (Oral presentation)

- D. Capela, A. Alves, V. Homem, L. Santos, From the shop to the drain - Volatile methylsiloxanes in cosmetics an 1 d personal care products, Environment International, Ms. Ref. No.: ENVINT-D-15-01732 (*under review*)

7 Limitations and Future Work

Despite the good results obtained and the main goals of this work have been achieved, there were some limitations, mainly related to time. Besides the fact that the GC-MS equipment be shared with other researchers, there was a period of time that the equipment was not working properly due to some technical problems, which led to the reschedule of experimental work and, consequently, to a delay.

Despite the low levels of contamination obtained with the use of a CP-Sil 8CB GC-MS capillary column (5% phenylmethylpolysiloxane in the coating) and a regular split/splitless injector with rubber septa, it should be interesting to test a low bleeding column and a Merlin Microseal injector, ideal for the analysis of siloxane compounds.

As future work, it will also be interesting to expand the range of siloxanes analysed to include the high molecular linear methylsiloxanes (L6 to L14), which are expected in several toiletry products. In order to complete the study, the range of products analysed may also be expanded. For example, with the inclusion of makeup products (liquid foundation, nail polish, etc.), specific day and night products (e.g. facial creams), perfumes, diaper creams, etc. Finally, the exposure to siloxanes by inhalation of these personal care products may also be investigated.

At this moment, two other papers related to the development and validation of the QuEChERS-GC/MS methodology to the analysis of VMSs in personal care products and to the occurrence and behaviour of these compounds in wastewater treatment plants are in preparation:

- D. Capela, A. Alves, V. Homem, L. Santos, A QuEChERS methodology coupled to GC–MS for the quantification of volatile methylsiloxanes in personal care products.

- D. Capela, V. Homem, N. Ratola, A. Alves, L. Santos, VMSs in the water and sludge lines of WWTPs – a review of levels and implications.

8 References

Abian, J. (1999). The coupling of gas and liquid chromatography with MS. *Journal of Mass Spectrometry, 34*(3), 157-163.

Agostino, M., Sanz, J., Sanz, M., Giuffrè, A., Sicari, V., Soria, A. (2015). Optimization of a solid-phase microextraction method for the gas chromatography-mass spectrometry analysis of blackberry (*rubus ulmifolius schott*) fruit volatiles. *Food Chemistry, 178*(3), 10-17.

Alvarino, T., Suarez, S., Katsou, E., Vazquez-Padin, J., Lema, J., Omil, F. (2015). Removal of pharmaceutical and personal care products from the sludge supernatant in a one stage nitritation/anammox process. *Water Research, 68*(2), 701-709.

Anastasiades, M., Lehotay, S., Stajnbaher, D., Schenck, F. (2003). *QuEChERS Methodology*. Available at: http://www.restek.com/pdfs/805-01-002.pdf [Accessed 23 March 2015]

Anastassiades, M. (2006). *The QuEChERS Method-Background information and recent developments*. Available at: http://www.eurl-pesticides.eu/library/docs/srm/1stws2006_lecture_anastassiades_quechers.pdf [Accessed 10 April 2015]

Barel, A., Paye, M., Maibach, H. (2014). *Handbook of Cosmetic Science and Technology* (4th ed.). Taylor & Francis.

Biesterbos, J., Beckmann, G., Wel, L., Anzion, R., Goetz, N., Dudzina, T., Scheepers, P. (2015). Aggregate dermal exposure to cyclic siloxanes in personal care products: implications for risk assessment. *Environment International, 74*(3), 231-239.

Biesterbos, J., Dudzina, T., Delmaar, C., Bakker, M., Russel, F., Von Goetz, N., Roeleveld, N. (2013). Usage patterns of personal care products: important factors for exposure assessment. *Food Chemistry Toxicology, 55*(8), 8-17.

Biomonitoring Program. (2008). *Consideration of Potential Designated Chemicals*. Available at: http://www.biomonitoring.ca.gov/sites/default/files/downloads/1208cyclosiloxanes.pdf [Accessed 15 March 2015]

Bletsou, A., Asimakopoulos, A., Stasinakis, A., Thomaidis, N., Kannan, K. (2013). Mass loading and fate of linear and cyclic siloxanes in a wastewater treatment plant in Greece. *Environmental Science and Technology, 47*(4), 1824-1832.

Bolzinger, M., Cogne, C., Lafferrere, L., Salvatori, F., Ardaud, P., Zanetti, M., Puel , F. (2007). Effects of surfactants on crystallization of ethylene glycol distearate in oil-in-water emulsion. *Colloids and Surfaces A: Physicochemical and Engineering Aspects, 299*(1-3), 93-100.

Brebbia, C., Eglite, M., Knets, I., Popov, V. (2011). *Environmental Health and Biomedicine* (1st ed.). United Kingdom: WIT press.

Bremmer, H., Lodder, L., Engelen, J. (2006). Cosmetics fact sheet. To assess the risks for the consumer. *Updated version for ConsExpo 4, RIVM*.

Brondi, S., Macedo, A., Vicente, G., Nogueira, A. (2011). Evaluation of the QuEChERS Method and Gas Chromatography–Mass Spectrometry for the Analysis Pesticide Residues in Water and Sediment. *Environmental Contamination and Toxicology, 86*(1), 18-22.

Brooke, D., Crookes, M., Gray, D., Robertson, S. (2005). *Environmental Risk Assessment Report: Dodecamethylcyclohexasiloxane* (2nd ed.). Bristol: Environment Agency.

Brunete, C., Miguel, E., Albero, B., Tadeo, J. (2010). Determination of cyclic and linear siloxanes in soil samples by ultrasonic-assisted extraction and gas chromatography-mass spectrometry. *Journal of Chromatography A, 1217*(45), 7024–7030.

Buser, A., Kierkegaard, A., Bogdal, C., MacLeod, M., Scheringer, M., Hungerbuhler, K. (2013). Concentrations in ambient aor and emissions of cyclic volatile methylsiloxanes in Zurich. *Environmental Science and Technology, 47*(13), 7045-7051.

Clark, C., Zytner, R., McBean, E. (2012). Analyzing volatile organic siloxanes in landfill biogas. *Canadian Journal of Civil Engineering, 39*(6), 667-673.

Companioni, E., Santos, F., Galceran, M. (2012). Analysis of linear and cyclic methylsiloxanes in water by headspace solid phase microextraction and gas chromatography-mass spectrometry. *Talanta, 89*(8), 63-69.

Cortada, C., Costa dos Reis, L., Vidal, L., Llorca, J., Canals, A. (2014). Determination of cyclic and linear siloxanes in wastewater samples by ultrasound-assisted dispersive liquid liquid microextraction followed by gas chromatography-mass spectrometry. *Talanta, 120*(46), 191-197.

Cortada, C., Vidal , L., & Canals, A. (2011). Determination of geosmin and 2-methylisoborneol in water and wine samples by ultrasound-assisted dispersive liquid-liquid microextraction coupled to gas chromatography-mass spectrometry. *Journal of Chromaography A, 1218*(1), 17-22.

Courant, F., Antignac, J., Maume, D., Monteau, D., Andre, F., Bizec, B. (2007). Determination of naturally occuring ostrogens and androgens in retail samples of milk and eggs. *Food Additives and Contaminants, 120*(20), 1358-1366.

CTFA (Cosmetic Toiletry and Fragrance Association). (2006). *International Cosmetic Ingredient Dictionary and Handbook* (11th ed.). Washington, DC: Color Additive Information.

Dauner, M., Sauer, U. (2000). GC-MS analysis of amino acids rapidly provides rich information for isotopomer balancing. *Biotechnology Progress, 16*(4), 642-649.

Dayan, N., Kromidas, L. (2011). *Formulating, Packaging, and Marketing of Natural Cosmetic Products* (1st ed.). New Jersey: John Wiley & Sons, Inc.

Dean, J. (2009). *Extraction Techniques in Analytical Sciences* (2nd ed.). (Wiley, Ed.) London: Analytical Techniques in the Sciences.

Dewil, R., Appels, L., Baeens, J., Buczynska, A., Vaeck, L. (2007). The analysis of volatile siloxanes in waste activated sludge. *Talanta, 74*(1), 14-19.

Dirtu, A., Eede, N., Malarvannan, G., Ionas, A., Covaci, A. (2012). Analytical methods for selected emerging contaminants in human matrices. *Analytical and Bioanalytical Chemistry, 404*(9), 2555-2581.

Douglas, F. (2011). GC/MC Analysis. *Scientific Testimony*: An online Journal, 1-9. Available at: http://www.scientific.org/information/about.html [Accessed 4 April 2015]

Dudzina, T., Goetz, N., Bogdal, C., Biesterbos, J., Hungerbuhler, K. (2014). Concentrations of cyclic volatile methylsiloxanes in European cosmetics and personal care products: Prerequisite for human and environmental exposure assessment. *Environment International, 62*, 86-94.

EC/HC. (2009). *(Environmental Canda/Health Canada) Screening assessment reports for the batch 2 challenge chemicals*. Available at: http://www.chemicalsubstanceschimiques.gc.ca/challenge-defi/batch-lot_2_e.htm [Accessed 24 November 2014]

Edser, C. (2015). Tip top condition: current trends in hair care formulation and latest ingredients. *Focus on Surfactants, 1*(1), 4-5.

Egmond, R., Sparham, C., Hastie, C., Gore, D., Chowdhury, N. (2013). Monitoring and modelling of siloxanes in a sewage treatement plant in the UK. *Chemosphere, 93*(5), 757-75.

Ellison , S., Rosslein, M., Williams, A. (2000). EURACHEM/CITAC Guide, Quantifying Uncertainty in Analytical Measurement . *Teddington*.

Environment Canada. (2012). Available at: https://ec.gc.ca/Publications/default.asp?lang=En&xml=253AE6E6-5E73-4AFC-81B7-9CF440D5D2C5 [Accessed 21 September 2015]

European Comission . (2014). Available at: http://ec.europa.eu/ [Accessed 19 October 2014]

European Parliament. (2009). Regulation (EC) N. 1223/2009 of the european parliament and of the Council of 30 November 2009 on cosmetic products. *Official Journal of the European Union, L 342/59*.

Evgenidou, E., Konstantinou, I., Lambropoulou, D. (2015). Occurrence and removal of transformation products of PPCPs and illicit drugs in wastewaters: A review. *Science of the Total Environment, 505*, 905-926.

EWG. (2012). *Environmental Working Group*. Available at: http://www.ewg.org/skindeep/ingredient/products/ [Accessed 15 December 2014]

Ezoddin, M., Majidi, B., Abdi, K. (2014). Evaluation of ultrasound-assisted in situ sorbent formation solid-phase extraction determination of arsenic in water, food and biological samples. *Environmental Technology, 36*(11), 1381-1388.

Ford, R. (1998). The human safety of the polycyclic musks AHTN and HHC in fragrances - a review. *Dtsch Lebensm Rundsch, 94*, 268-275.

Genualdi, S., Harner, T., Cheng, Y., MacLeod, M., Hansen, K., Van Egmond, R., Chi Lee, S. (2011). Global distribution of linear and cyclic volatile methyl siloxanes in air. *Environmental Science and Technology, 45*(8), 3349-3354.

Goddard, E., Gruber, J. (1999). Principles of Polymer Science and Technology in Cosmetics and Personal Care. *Cosmetic Science and Technology Series, 22*, Marcel Dekker Inc. .

Golovko, O., Kumar, V., Fedorova, G., Randak, T., Grabic, R. (2014). Seasonal changes in antibiotics, antidepressants/psychiatric drugs, antihistamines and lipid regulators in a wastewater treatment plant. *Chemosphere, 111*, 418-426.

Gouin, T., Egmond, R., Sparham, C., Hastie, C., Chowdhury, N. (2013). Simulated use and wash-off release of decamethylcyclopentasiloxane used in antiperspirants. *Chemosphere, 93*(5), 726-734.

Groz, M., Bueno, M., Rosain, D., Fenet, H., Casellas, C., Pereira, C., Gomez, E. (2014). Detection of emerging contaminants (UV filters, UV stabilizers and musks) in marine mussels from Portuguese coast by QuEChERS extraction and GC–MS/MS. *Science of The Total Environment, 493*(13), 162-169.

Háková, E., Vrkoslav, V., Míková, R., Pecková, K., Bosáková, Z., Cvcka, J. (2015). Localization of double bonds in tricyglycerols using high-performance liquid chromatography/atmospheric pressure chemical ionization ion-trap mass spectrometry. *Analytical and Bioanalytical Chemistry, 407*(17), 5175-5188.

Hanssen, L., Warner, N., Braathen, T., Odland, T., Lund, E., Nieboer, E., Sandanger, T. (2013). Plasma concentrations of cyclic volatile methylsiloxanes (cVMS) in pregnant and postmenopausal Norweigian womn and self-reported use of personal care products (PCPs). *Environment International, 51*, 82-87.

Harris, D. (2003). *Quantitative Chemical Analysis* (6th ed.). New York: W.H Freeman & Company.

Hill, L. (2009). *Ion Trap*. Available at: https://www.jic.ac.uk/services/metabolomics/topics/lcms/iontrap1.htm [Accessed 3 March 2015]

Hinshaw, J., Taylor, T., Walsh, D. (2009). *Split/splitless injection for capillary GC*. Available at: http://www.chromacademy.com/Essential_Guide_Webcast/Split_Splitless_Injection/Split_Splitless_Injection.pdf [Accessed 25 May 2015]

Holson, J., Reynolds, J. (1997). *An inhalation range-finding reproductive toxicity study of octamethylcyclotetrasiloxane (D4) in male rats, document control no. 86980000049.* Washington, DC: Toxic substance control act public docket office.

Homem, V., Silva, J., Cunha, C., Alves, A., Santos, L. (2013). New analytical method for the determination of musks in personal care products by Quick, Easy, Cheap, Effective, Rugged, and Safe extraction followed by GC-MS. *Journal of Separation Science, 36*(13), 2176-2184.

Hong, W., Jia, H., Liu, C., Zhang, Z., Sun, Y., Li, Y. (2014). Distribution, source, fate and bioaccumulation of methyl siloxanes in marine environment. *Environmental Pollution, 191*, 175-181.

Horii, Y., Kannan, K. (2008). Survey of organosilicone compounds, includig cyclic and linear siloxanes, in personal-care and household products. *Environmental Contamination and Toxicology, 55*(4), 701-710.

Hubschmann, H. (2015). *Handbook of GC-MS: Fundamentals and Applications* (3rd ed.). Wiley-VCH Verlag GmbH & Co. kGaA.

INCI. (2000). *International Nomenclature of Cosmetic Ingredients* . Available at: http://www.cirs-reach.com/Cosmetic_Inventory/International_Nomenclature_of_Cosmetic_Ingredients_INCI.html [Accessed 23 February 2015]

ISO. (2006). *International Vocabulary of Basic and General Terms in Metrology* (3rd ed.). Geneva, Switzerland.

IUPAC. (1997). *Compendium of Chemical Terminology (the "Gold Book). Complied by A. D. McNaught and A. Wilkinson* (2nd ed.). Oxford : Blackwell Scientific Publications.

IUPAC. (2006). *Compendium of Chemical Terminology* (2nd ed.). Wiley.

Jalbani, N., Yilmaz, E., Alosmanov, R., Soylak, M. (2014). Solid-phase extraction of copper and zinc in water samples using diethylamine-modified phosphorus-containing polymer. *Desalination and Water Treatment, 93*, 435-444.

Jawaid, S., Talpur, F., Nizamani, S., Memon, N., Afridi, H., Khaskheli, A. (2014). Rapid in situ esterification method for the determination of banzoic acid in dairy milk by GC-FID. *Food Analytical Methods, 8*(6), 1477-1483.

Jia, H., Zhang, Z., Wang, C., Hong, W., Sun, Y., Li, Y. (2015). Trophic transfer of methyl siloxanes in the marine food web from coastal area of northern China. *Environmental Science and Technology, 49*, 2833-2840.

Jiao, T., Li, C., Zhuang, X., Cao, S., Chen, H., Zang, S. (2015). The new liquid-liquid extraction method for separation of phenolic compounds from coal tar. *Chemical Engineering Journal, 266*, 148-155.

Johnson, W., Bergfeld, W., Belsito, D., Hill, R., Klaassen, C., Liebler, D., Andersen, F. (2012). Safety Assessment of Cyclomethicone, Cyclotetrasiloxane, Cyclopentasiloxane, Cyclohexasiloxane, and Cycloheptasiloxane. *International Journal of Toxicology, 30*, 149-227.

Jovanovic, M., McMahon, J., McNett, D., Tobin, J., Plotzke, K. (2008). In vitro and in vivo percutaneous absorption of 14c-octamethylcyclotetrasiloxane (14C-D4) and 14C-decamethylcyclopentasiloxane (14C-D5). *Regulatory Toxicology and Pharmacology,* 50 (2), 239-248.

Kaj, L., Anderson, J., Cousins, A., Remberger, M., Cato, I. (2005). *Results from the Swedish National Screening Programme 2004 Subreport 4: Siloxanes*. Available at: http://www.imm.ki.se/Datavard/PDF/B1643_siloxaner.pdf [Accessed 15 April 2015]

Kierkegaard, A., McLachlan, M. (2013). Determination of linear and cyclic volatile methylsiloxanes in air at a regional background site in Sweden. *Atmospheric Environment, 80*, 322-329.

Kim, N., Chun, S., Cha, D., Kim, C. (2013). Determination of Siloxanes in Biogas by Solid-phase Adsorption on Active Carbon. *Korean Chemistry Society, 34*(8), 2353-2357.

Kozerski, G., Xu, S., Miller, J., Durham, J. (2014). Determination of soil-water sorption coefficients of volatile methylsiloxanes. *Environmental Toxicology and Chemistry, 33*(9), 1937-1945.

Lacaze, J., Stobo, L., Turrell, E., Quilliam, M. (2007). Solid-phase extraction and liquid chromatography-mass spectrometry for the determination of free fatty acids in shellfish. *Journal of Chromatography A, 1145*(1-2), 51-57.

Lassen, C., Hansen, C., Mikkelsen, S., Maag, J. (2005). *Siloxanes-consumption, toxicity and alternatives* (1st ed., Vol. No 1031). Denmark : COWI A/S.

Lees, M. (2011). *Skin Care: Beyond the Basis* (4th edition ed.). Cengage Learning.

Lide, D. (2007). *CRC Handbook of Chemistry and Physics. In: B. Raton*, 88th edition. New York : CRC Press, Taylor & Francis, pp. 3-224

Lockwood, D. (2015). *Siloxanes unexpectedly observed in antarctic soil and marine life*. Available at: http://cen.acs.org/articles/93/web/2015/02/Siloxanes-Unexpectedly-Observed-Antarctic-Soil.html [Accessed 29 May 2015]

Lorenz, C., Von Goetz, N., Schringer, M., Wormuth, M., Hungerbuhler, K. (2011). Potential exposure of German consumers to engineered nanoparticles in cosmetics and personal care products. *Nanotoxicology, 5*, 12-29.

Lu, Y., Yaun, T., Wang, W., Kannan, K. (2011). Concentrations and assessment of exposure to siloxanes and synthetic musks in personal care products from China. *Environmental Pollution, 159*(12), 3522-3528.

MacLeod, M., Kierkegaard, A., Genualdi, S., Harner, T., Scheringer, M. (2013). Junge relationships in measurement data for cyclic siloxanes in air. *Chemosphere, 93*(5), 830-834.

March, R. (2000). Quadrupole ion trap mass spectrometry: a view ar the turn of the century. *International Journal of Mass Spectrometry, 200*(1-3), 285-312.

McClellan, K., Halden, R. (2010). Pharmaceuticals and personal care products in archived US biosolids from the 2001 EPA national sewage sludge survey. *Water Resources, 44*, 658-668.

McKim, J., Wilga, P., Breslin, W., Plotzke, K., Gallavan, R., Meeks, R. (2001a). Potencial estrogenic and antiestrogenic activity of the cyclic siloxane octamethylcyclotetrasiloxane (D4) and linear siloxane hexamethyldisiloxane (HMDS) in immature rats using the uterotrophic assay. *Toxicological Sciences, 63*(1), 37-46.

McKim, J., Kolesar, G., Jean, P., Meeker, L., Wilga, P., Schoonhoven, R., Meeks, R. (2001b). Repeated inhalation exposure to octamethylcyclotetrasiloxane producs hepatomegaly, transient hepatic hyperplasia, and sustained hypertrophy in female fischer 344 rats in a manner similar to phenobarbital. *Toxicology and Applied Pharmacology, 172*, 83-92.

Meeks, R., Stump, D., Siddiqui, W., Holson, J., Plotzke, K., Reynolds, V. (2007). An inhalation reproductive toxicity study of octamethylcyclotetrasiloxane (D4) in female rats using multiple and single day exposure regimens. *Reproductive Toxicology, 23*, 192-201.

Meffe, R., Bustamante, I. (2014). Emerging organic contaminants in surface water and groundwater: A first overview of the situation in Italy. *Science of The Total Environment, 481*, 280-295.

Montemayor, B., Price, B., Egmond, R. (2013). Accounting for intended use application in characterizing uthe contributions of cyclopentasiloxane (D5) to aquatic loadings following personal care product use: antiperspirants, skin care products and hair care products. *Chemosphere, 93*(5), 735-740.

Nakata, H., Hinosaka, M., Yanagimoto, H. (2015). Macrocyclic-, polycyclic-, and nitro musks in cosmetics, household commodities and indoor dusts collected from Japan: implications for their human exposure. *Ecotoxicology and Environmental Safety, 111*, 248-255.

Neyens, E., Baeyens, J., Dewil, R., De heyder, B., Hazerd, J. (2004). Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering. *Journal of Hazardous Materials, 106*(2-3), 83-92.

OECD. (2007). *Manual for Investigation of HPV Chemicals*. Available at: http://www.oecd.org/document/7/0,3343,en_2649_34379_1947463_1_1_1_1,00.html [Accessed 15 October 2014]

Oosterhuis, M., Sacher, F., Laak, T. (2013). Prediction of concentration levels of metformin and other high consumption pharmaceuticals in wastewater and regional surface water based on sales data. *Science of the Total Environment, 442*, 380-388.

Pacchiarotta, T., Nevedomskaya, E., Carrasco-Pancorbo, A., Deelder, A., Mayboroda, O. (2010). Evaluation of GC-APCI/MS and GC-FID as a complementary platform. *Journal of Biomolecular Techniques, 21*, 205-213.

Paller, A., Hawk, J., Honig, P., Giam, Y., Hoath, S., Mack, M., Stamatas, G. (2011). New insights about infant and toddler skin: implications for sun protection. *Pediatrics, 128*(1).

Park, J., Choi, J., El-Aty, A., Kim, A. (2011). Simultaneous multiresidue analysis of 41 pesticide residues in cooked foodstuff using QuEChERS: comparison with classical method. *Food Chemistry, 128*(1), 241-253.

Picó, Y. (2008). *Food contaminants and residue analysis* (1st ed.). Amsterdam: Elsevier.

Pierce, J. (2004). *Siloxanes in Landfill and Digester Gas Update*. Available at: http://www.scsengineers.com/scs-white-papers/siloxanes-in-landfill-and-digester-gas-update [Accessed 20 May 2015]

Pieri, F., Katsoyiannis, A., Martellii, T., Hughes, D., Jones, K., Cincinelli, A. (2013). Occurence of linear and cyclic volatile methyl siloxanes in indoor air samples (UK and Italy) and their isotopic characterization. *Environment International, 59*, 363-371.

Power, C. (2010). Cosmetics, identity and consciousness. *Journal of Consciousness Studies, 17*, 73-94.

Quemet, A., Maloubier, M., Dalier, V., Ruas, A. (2014). Development of an analysis method of minor uranium isotope ratio measurements using electron multipliers in Thermal Ionization Mass Spectrometry. *International Journal of Mass Spectrometry, 374*, 26-32.

Quinn, A., Regan, J., Tobin, J., Marinik, B., McMahon, M. D., Sushynski, C., Plotzke, K. (2007). In vitro and in vivo evaluation of the estrogenic, androgenic, and progestogenic potential of two cyclic siloxanes. *Toxicological Sciences , 96*, 145-153.

Raich-Montiu, J., Ribas-Font, C., Arespacochaga, N., Roig-Torres, E., Broto-Puig, F., Crest, M., Cortina, J. (2014). Analytical methodology for sampling and analysing eight siloxanes and trimethylsilanol in biogas from different wastwater treatment plants in Europe. *Analytica Chimica Acta, 812*, 83-91.

Ramírez, N., Borrull, F., Marcé, R. (2012). Simultaneous determination of parabens and synthetic musks in water by stir-bar sorptive extraction and thermal desorption-gas chromatography-mass spectrometry. *Journal of Separation Science, 35*(4), 580-588.

Ratola, N., Santos, L., Herbert, P., Alves, A. (2006). Uncertainty associated to the analysis of organochlorine pesticides in water by solid-phase microextraction/gas chromatography-electron capture detection - Evaluation using two different approaches. *Analytica Chimica Acta,* 573-574, 202-208.

Reddy, M., Looney, R., Utell, M., Plotzke, K., Andersen, M. (2007). Modeling of human dermal absorption of octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5). *Toxicological Science, 92*(2), 422-431.

Regueiro, J., llompart, M., Jares, C., Monteagudo, G., Cela, R. (2008). Ultrasound-assisted emulsification-microextraction of emergent contaminants and pesticides in environmental waters. *Journal of Chromatography A, 1190*(1-2), 27-38.

Ribeiro, C., Ribeiro, A., Maia, A., Gonçalves, V., Tiritan, M. (2014). New trends in sample preparation techniques for environmental analysis. *Critical Reviews in Analytical Chemistry, 44*(2), 142-185.

Romanowski, P. (2014). *A Cosmetic Industry Overview for Cosmetic Chemists*. Available at: http://chemistscorner.com/a-cosmetic-market-overview-for-cosmetic-chemists/ [Accessed 24 May 2015]

Sanchís, J., Martínez, E., Ginebreda, A., Farré, M., Barceló, D. (2013). Occurrence of linear and cyclic volatile methylsiloxanes in wastewater, surface water and sediments from Catalonia. *Science of the Total Environment, 443*, 530-538.

Sapozhnikova, Y., Lehotay, S. (2013). Multi-class, multi-residue analysis of pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers and novel flame retardants in fish using fast, low-pressure gas chromatography-tandem mass spectrometry. *Analytica Chimica Acta, 758*, 80–92.

SCCP. (2005). *(Scientific Committee on Consumer Products). Opinion on octamethylcyclotetrasiloxane (D4) cyclomethicone (INCI name)*. Available at: http://ec.europa.eu/health/archive/ph_risk/committees/04_sccp/docs/sccp_o_035.pdf [Accessed 12 April 2015]

SCCS (2010). (Scientific Commitee on Consumer Safety). *Opinion on Cyclomethicone - Octamethylcyclotetrasiloxane (Cyclotetrasiloxane, D4) and Decamethylcyclopentasiloxane (Cyclopentasiloxane, D5). SCCS/1241/10.* Available online at http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_029.pdf, 2010. [last accessed on 28.12.2015].

SCCS (2012). (Scientific Commitee on Consumer Safety). *The SCCS'S Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation* (8th ed.). Brussels.

Schuur, B., Verkuijl, B., Minnaard, A., Vries, J., Feringa, B. (2011). Chiral separation by enantioselective liquid-liquid extraction. *Organic and Biomolecular Chemistry, 9*(48), 36-51.

SEHSC. (2009). *(Silicones Environmental, Health and Safety Council of North America), Decamethylcyclopentasiloxane (D5)*. Available at: http://www.dowcorning.com.cn/zh_CN/content/about/aboutehs/EHSPortalFiles/GPS_Safety_Report_541-02-6_D5.pdf [Accessed 15 April 2015]

Simpson, N. (2000). *Solid-Phase Extraction Principles, Techniques, and Application* (1st ed.). California: Marcel Dekker.

Skoog, D., Holler, F., Crouch, S. (2007). *Principles of Intrumental Analysis* (6th ed.). Belmont, USA: Thomson Brooks/Cole.

Somsen, G., Jong, G. (2002). Multidimensional chromatography; biomedical and pharmaceutical applications. Chichester, UK: John Wiley & Sons Ltd.

Sparham, C., Egmond, R., O'Connor, S., Hastie, C., Whelan, M., Kanda, R., & Franklin, O. (2008). Determination of decamethylcyclopentasiloxane in river water and final effluent by headspace gas chromatography/mass spectrometry. *Journal of Chromatography A, 1212*(1-2), 124-129.

Sparkman, D., Penton, Z., & Kitson, F. (2011). *Gas chromatography and mass spectrometry: a practical guide* (2nd edition ed.). Oxford: Elsevier Science and Technology .

Statistics Portal. (2008). *Statistics Portal*. Available at: http://www.statista.com/topics/1008/cosmetics-industry/ [Accessed 17 October 2014]

Stump, D., Reynolds, D. (1997). *An inhalation range-finding reproductive toxicity study of octamethylcyclotetrasiloxane (D4) in male rats, document control no. 86980000061*. Washington, DC: toxic substance control act public docket office.

Tanase, I., Popa, D., Udristioiu, G., Bunaciu, A., Aboul-Enein, H. (2015). Estimation of the uncertainty of the measurement results of some trace levels elements in document paper samples using ICP-MS. *RSC Advances,* 5(15), 11445-11457.

Tanoue, R., Nomiyamaa, K., Nakamura, H., Hayashi, T., Kima, J., Isobea, T., Tanabea, S. (2014). Simultaneous determination of polar pharmaceuticals and personal care products in biological organs and tissues. *Journal of Chromatography A, 1355*, 193-205.

Teasdale, A., Ulman, K., Domoradzki, J., Walsh, P. (2015). Establishing limits for dermal absorption of elemental impurities. *Pharmaceutical Technology, 39*(9).

The Personal Care Products Council . (2014). Available at: http://www.personalcarecouncil.org [Accessed 22 March 2015]

UK EA. (2009). *UK Environmental Agency*. Available at: http://cdn.environmentagency. gov.uk/scho0309bpqz-e-e.pdf [Accessed 15 December 2014]

US EPA. (2007). *US EPA*. Available at: http://www.epa.gov/chemrtk/pubs/update/spnchems.htm [Accessed 24 October 2014]

Vidal, L., Psillakis, E., Domini, C., Grané, N., Marken, F., Canals, A. (2007). An ionic liquid as a solvent for headspace single drop microextraction of chlorobenzenes from water samples. *Analytica Chimica Acta, 584*(1), 189-195.

Viegas, R., Afonso, C., Crepo, J., Coelhosa, I. (2007). Modelling of the enantio-selective extraction of propranolol in a biphasic system. *Separation and Purification Technology, 53*(3), 224-234.

Wang, D., Aggarwal, M., Tait, T., Brimble, S., Pacepavicius, G., Kinsman, L., Alaee, M. (2015b). Fate of anthropogenic cyclic volatile methylsiloxanes in a wastewater treatment plant. *Water Research, 72*, 209–217.

Wang, D., Du, J., Pei, W., Liu, Y., Gup, M. (2015a). Modeling and monitorin cyclic and linear volatile methylsiloxanes in a wastewater treatement plant using constant water level sequencing batch reactors. *Science of The Total Environment, 512-513*, 472-479.

Wang, D., Steer, H., Tait, T., Williams, Z., Pacepavicius, G., Young, T., Alaee, M. (2013). Concentrations of cyclic volatile methylsiloxanes in biosolids amended soil, influent, effluent, receiving water, and sediment of wastewater treatment plants in Canada. *Chemosphere, 93*(5), 766-773.

Wang, R., Moody, R., Koniecki, D., Zhu, J. (2009). Low molecular weight cyclic volatile methylsiloxanes in cosmetic products sold in Canada: implication for dermal exposure. *Environment International, 35*(6), 900-904.

Warner, N., Evenst, A., Chistensen, G., Gabrielsen, G., Borga, K., Leknes, H. (2010). Volatile siloxanes in European Arctic: assessment of sources and special distribution. *Environmental Science and Technology, 44*, 7705-10.

Weissermel, K., Arpe, H. (2003). *Industrial Organic Chemistry* (4th ed.). Frankfurt: Wiley VCH.

Werme, C. (2010). *Pharmaceuticals and personal care products*. Available at: http://www.sfei.org/sites/default/files/general_content/PPCP_profile_0.pdf [Accessed 20 April 2015]

Whelan, M., Egmond, R., Gore, D., Sanders, D. (2010). Dynamic multi-phase partitioning of decamethylcyclopentasiloxane (D5) in river water. W*ater Research, 44*(12), 3679-3686.

Xu, L., Shi, Y., Cai, Y. (2013). Occurrence and fate of volatile siloxanes in a municipal wastewater treatment plant of Beijing, China. *Water Research, 47*(2), 715-724.

Xu, L., Shi, Y., Liu, N., Cai, Y. (2015). Methyl siloxanes in environmental matrices and human plasma/fat from both general industries and residential areas in China. *Science of the Total Environment, 505*, 454-463.

Yebra, M., Cancela, S., Cespon, R. (2008). Automatic determination of nickel in food by flame atomic absorption spectrometry. *Food Chemistry, 108*(2), 774-778.

Yucuis, R., Stanier, C., & Hornbuckle, K. (2013). Cyclic siloxanes in air, including identification of high levels in Chicago and distinct diurnal variation. *Chemosphere, 92*(8), 905–910.

Zenobio, J. (2015). Presence and effects of pharmaceutical and personal care products on the Baca National Wildlife Refuge. *Chemosphere, 120*, 750-755.

Zhang, Z., Qi, H., Ren, N., Li, Y., Gao, D., Kannan, K. (2011). Survey of cyclic and linear siloxanes in sediment from the Songhua River and in sewage sludge from wastewater treatment plants, Northeastern China. *Environmental Contamination and Toxicology, 60*(2), 204-211.

Zhao, P., Huang, B., Han, Y., Zou, N., Gu, K. (2014). Rapid multiplug filtration cleanup with multiple-walled carbon nanotubes and gas chromatography-triple-quadrupole mass spectrometry detection for 186 pesticide residues in tomato and tomato products. *Journal of Agricultural and Food Chemistry, 62*, 3710-3725.

Zhu, Y., Wang, J., Li, X., Zhao, D., Sun, J., Liu, X. (2015). Self-assembly and emulsification of dopamine-modified hyaluronan. *Carbohydrate Polymers, 123*(25), 72-79.

Appendix 1. GC-MS chromatograms

In Figures A1 and A2 are represented the chromatograms in SIS mode of a body lotion spiked with a 500 mg.L^{-1} mix siloxanes standard and the chromatogram for the same body lotion without spike, respectively.

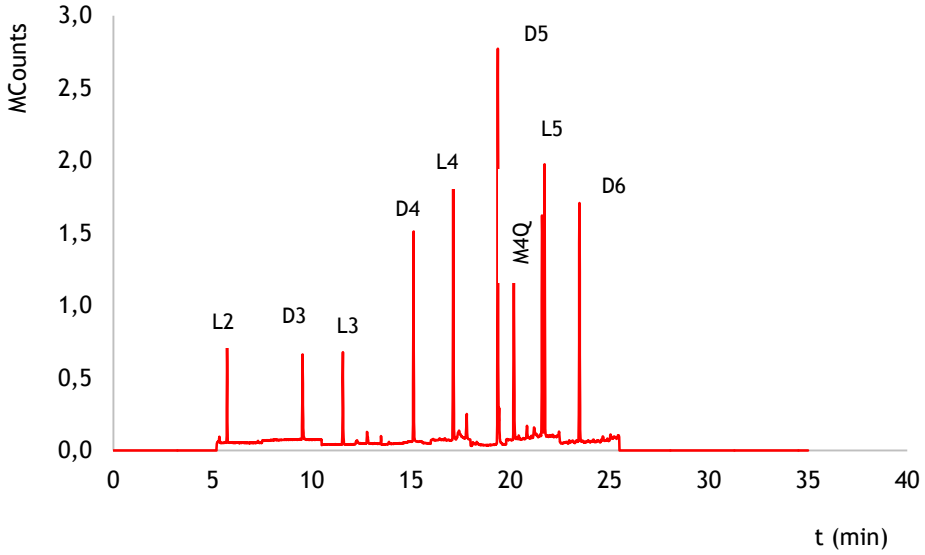


Figure A1: Chromatogram in SIS mode of an extracted sample of body lotion spiked with a 500 mg.L^{-1} mix siloxanes standard prepared in hexane.

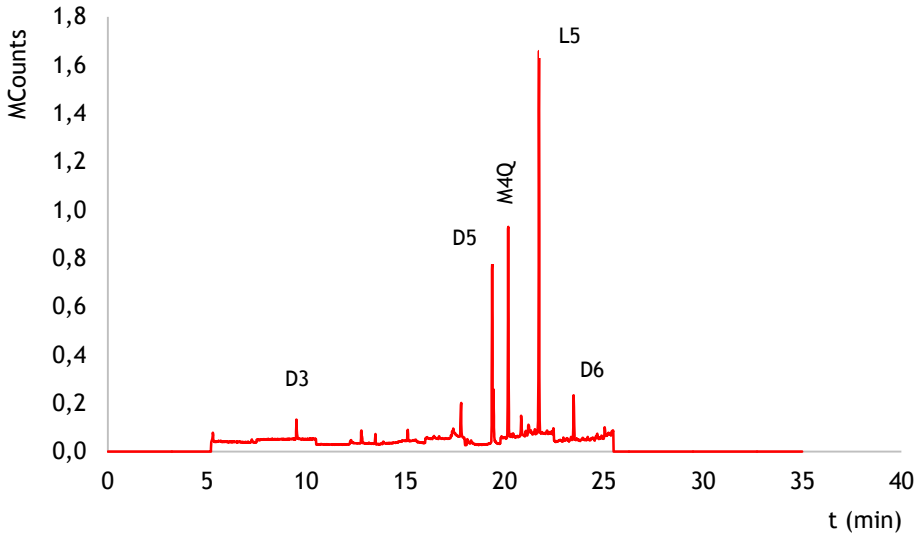


Figure A2: Chromatogram in SIS mode of an extracted sample of body lotion.

Appendix 2. Calibration curves

The average response factors (RF) obtained for each siloxane at different concentration levels and used to construct the calibration curves are shown in Table A1. The calibration curves for each compound are represented in Figure A3 to Figure A10. The calibration curves equations and their respective validation parameters are shown in Table A2.

Table A1: Response factors (RF) obtained for each siloxane.

C ($mg.L^{-1}$)	RF							
	L2	L3	L4	L5	D3	D4	D5	D6
0.005	0.020	0.014	0.016	0.013	0.040	0.032	0.047	0.031
0.010	0.035	0.021	0.037	0.025	0.054	0.039	0.065	0.044
0.050	0.190	0.122	0.231	0.126	0.142	0.181	0.213	0.134
0.100	0.457	0.216	0.377	0.207	0.213	0.299	0.341	0.213
0.250	0.963	0.537	1.058	0.565	0.513	0.704	0.847	0.533
0.500	1.426	0.865	1.942	1.193	0.779	1.349	1.567	1.062
0.750	2.203	1.331	3.091	1.895	1.224	2.017	2.458	1.690
1.000	2.997	1.835	4.365	2.608	1.648	2.765	3.425	2.260
2.000	5.701	3.635	9.024	5.402	3.356	5.489	6.999	4.386
2.500	6.801	4.331	10.663	6.332	4.005	6.439	8.329	4.954

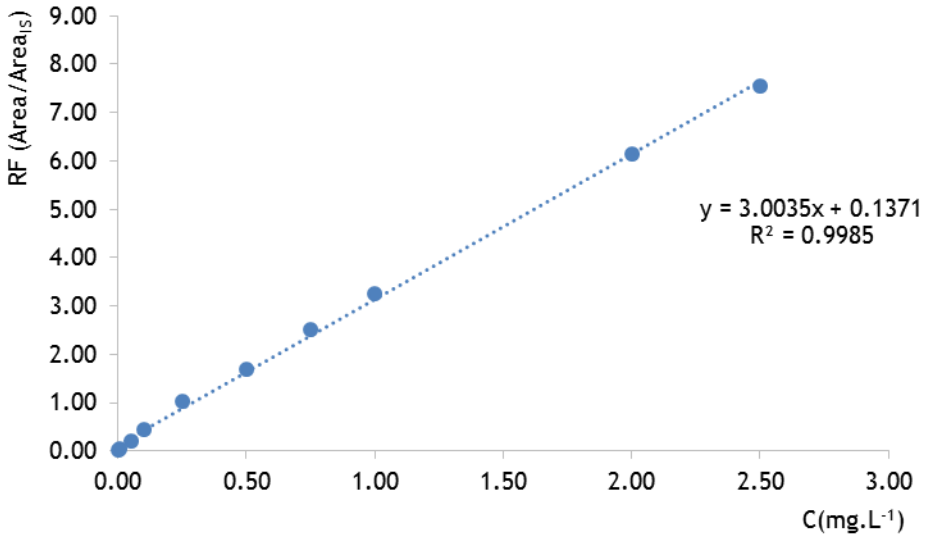


Figure A3: Calibration curve of L2.

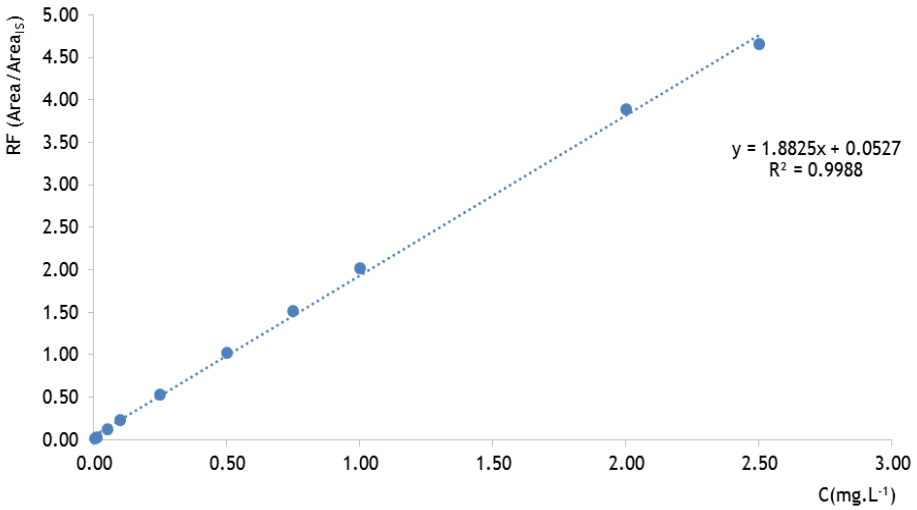


Figure A4: Calibration curve of L3.

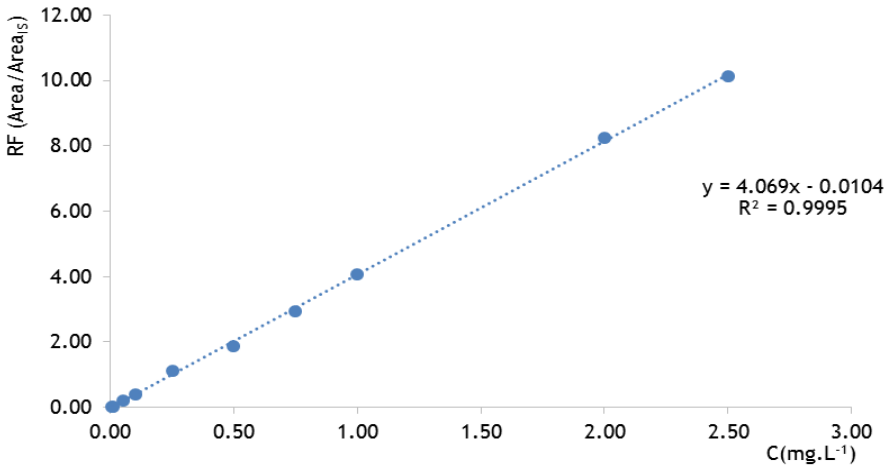


Figure A5: Calibration curve of L4.

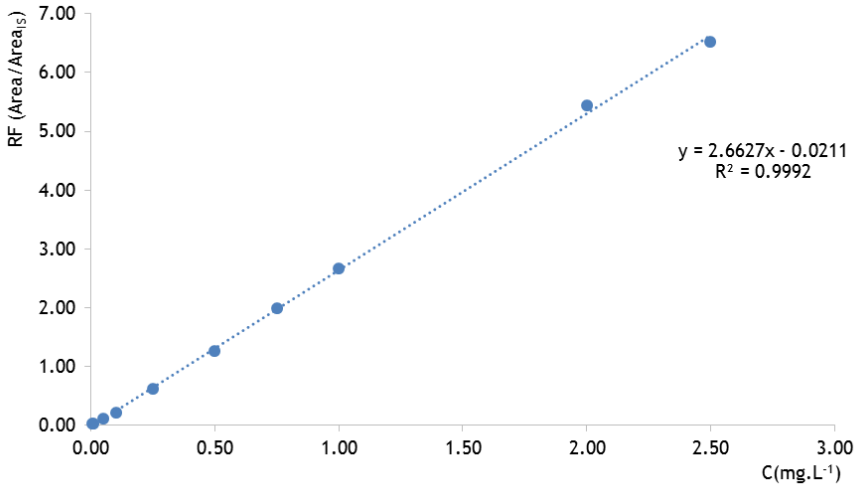


Figure A6: Calibration curve of L5.

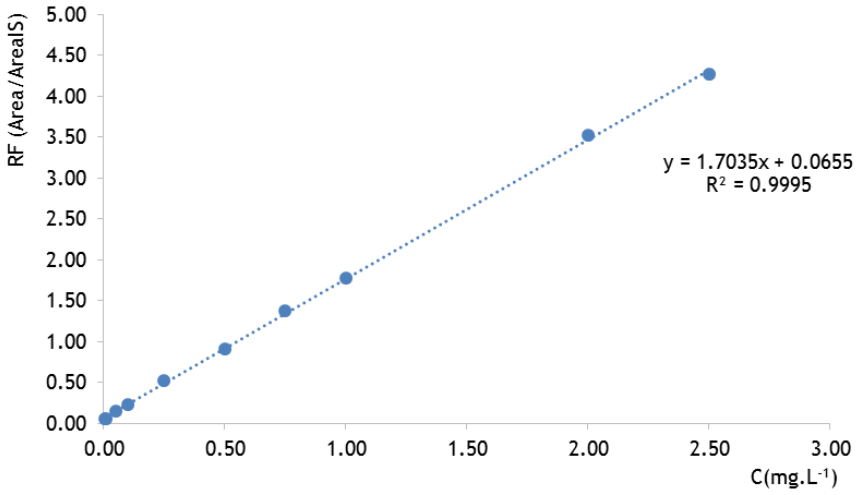


Figure A7: Calibration curve of D3.

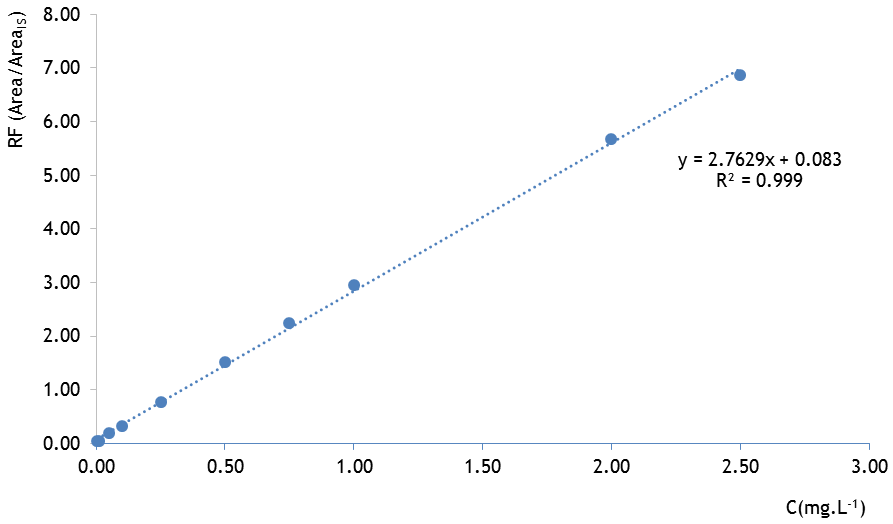


Figure A8: Calibration curve of D4.

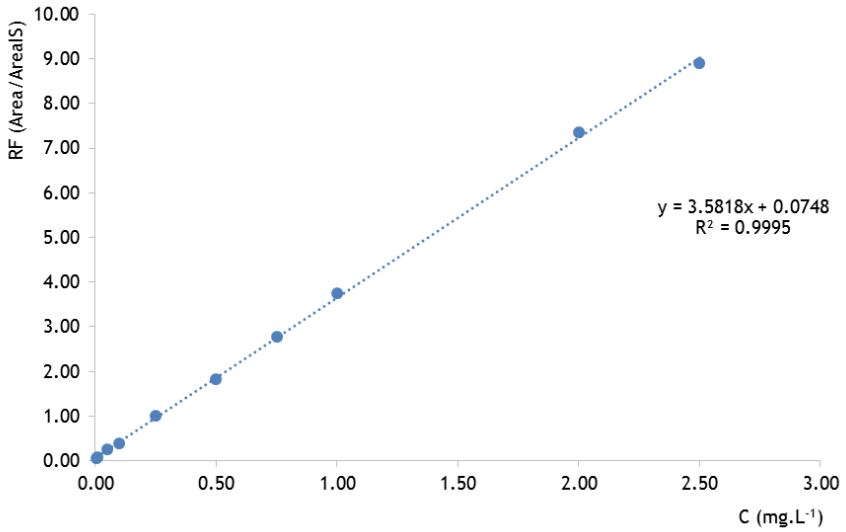


Figure A9: Calibration curve of D5.

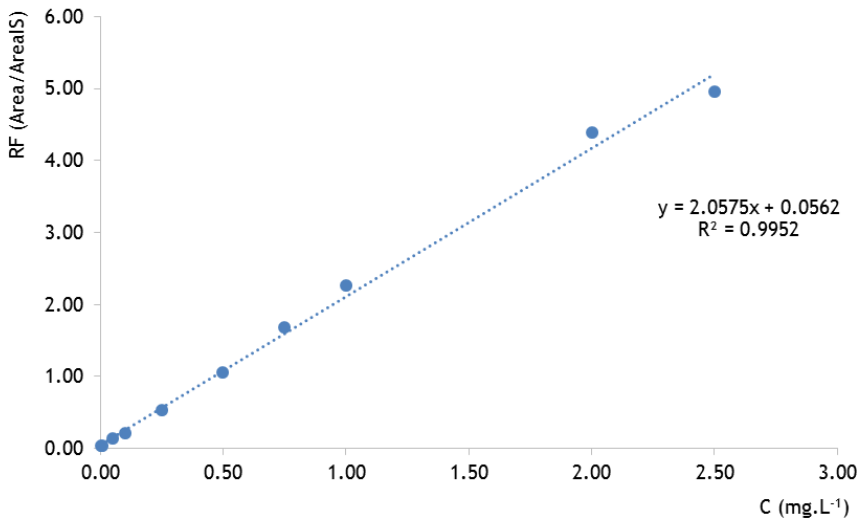


Figure A10: Calibration curve of D6.

Table A2: Calibration curves equations and their respective validation parameters.

Compound	Calibration curve equation	R > 0.995	Sa/a (< 5%)	$\frac{b-Sb}{b+Sb}$	b-Sb < 0 < b+Sb
L2	RF = (3.00±0.04)C + (0.14±0.05)	0.9985 ✓	1.37% ✓	$\frac{0.092}{0.183}$	✖
D3	RF = (1.70±0.01)C + (0.07±0.02)	0.9995 ✓	0.81% ✓	$\frac{0.050}{0.081}$	✖
L3	RF = (1.88±0.02)C + (0.05±0.03)	0.9988 ✓	1.22% ✓	$\frac{0.027}{0.078}$	✖
D4	RF = (2.76±0.03)C + (0.08±0.03)	0.999 ✓	1.09% ✓	$\frac{0.050}{0.116}$	✖
L4	RF = (4.07±0.03)C + (-0.01±0.03)	0.9995 ✓	0.69% ✓	$\frac{-0.039}{0.018}$	✓
D5	RF = (3.58±0.03)C + (0.07±0.03)	0.9995 ✓	0.76% ✓	$\frac{0.045}{0.105}$	✖
L5	RF = (2.66±0.03)C + (-0.02±0.03)	0.9992 ✓	0.97% ✓	$\frac{-0.050}{0.007}$	✓
D6	RF = (2.06±0.05)C + (0.06±0.06)	0.9952 ✓	2.46% ✓	$\frac{0.000}{0.112}$	✖

Appendix 3. Determination of global uncertainty

Uncertainty associated to the standard preparation, U_1

The uncertainty associated to the standard preparation was determined with the following equation:

$$U_1 = \sqrt{\sum_i \left(\frac{\Delta m_i}{m_i}\right)^2} \quad \text{(A1)}$$

wherein Δm_i represents the error associated to the equipment/measurement material and m_i represents the value measured by the equipment/measurement material. The uncertainty associated to the equipment was estimated based on a triangular distribution.

Uncertainty associated to the calibration curve, U_2

The uncertainty associated to the calibration curve was determined by the ratio between the standard deviation of a concentration, S_{x0}, and the concentration, x_0.

$$U_2 = \frac{S_{x0}}{x_0} = \frac{1}{x_0}\frac{S_{y/x}}{a}\sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(y_o - \bar{y})^2}{a^2 \sum_i (x_i - \bar{x})^2}} \quad \text{(A2)}$$

wherein $S_{y/x}$ corresponds to the standard deviation, a represents the slope of the linear regression, m the number of replicates performed, n the number of the standards used in the calibration curve, y_0 corresponds to the value of the area for each x_i, $\bar{y}$ represents the average of the values of y_i, x_i corresponds to the concentration of the standard i used in the calibration curve, and $\bar{x}$ corresponds to the average of the values of x_i.

Uncertainty associated to the precision, U_3

The value of the uncertainty associated to the precision was calculated based of the following equation:

$$U_3 = \frac{s}{y_{med}\sqrt{n}} \quad \text{(A3)}$$

wherein s corresponds to the standard deviation from the precision assays performed, y_{med} represents the value of the average area of the assays for a given concentration, and n corresponds to the number of assays.

Uncertainty associated to the accuracy, U_4

The uncertainty associated to the accuracy is determined in a certain way similar to the uncertainty associated to the precision:

$$U_4 = \frac{s(\eta)}{\sqrt{n}} \quad \text{(A4)}$$

wherein $s(\eta)$ corresponds to the standard deviation of the average percentage of recovery, and n represents the number of assays.

Global uncertainty, U

The global uncertainty is determined by the square root of the sum of squares of each of the uncertainties mentioned before:

$$U = \sqrt{U_1{}^2 + U_2{}^2 + U_3{}^2 + U_4{}^2} \quad \text{(A5)}$$

Variation of the relative weight of each individual source of uncertainty

Figure A11: Relative weight of each individual source of uncertainty on moisturizers.

Figure A12: Relative weight of each individual source of uncertainty on shower gels.

Figure A13: Relative weight of each individual source of uncertainty on deodorants.

Figure A14: Relative weight of each individual source of uncertainty on toothpastes.

Figure A15: Relative weight of each individual source of uncertainty on solid soap.

Figure A16: Relative weight of each individual source of uncertainty on shampoos.

Figure A17: Relative weight of each individual source of uncertainty on hair conditioner.

Figure A18: Relative weight of each individual source of uncertainty on aftershaves.

Figure A19: Relative weight of each individual source of uncertainty on shaving foams.

Appendix 4. Information present in the label of the products analysed

Table A3. Information present in the label of the products selected for the analysis of VMSs content.

Product Category	Product Subcategory	Code	cVMSs presence (as written in the ingredients list)
Dentifrice products	Toothpastes adult	A1	None
		A2	None
		A3	None
		A4	None
		A5	None
		A6	None
	Toothpastes children	A7	None
		A8	None
		A9	None
		A10	None
		A11	None
		A12	None
Toilet soaps	Solid soap	B1	None
		B2	None
		B3	None
		B4	None
		B5	None
		B6	None
		B7	None
		B8	None
		B9	None
	Gel soap	C1	None
		C2	None
		C3	None
		C4	?
		C5	None
		C6	None
Moisturizers	Hand cream	D1	Dimethicone
		D2	Dimethicone
		D3	Dimethicone
	Adult body lotion/milk/cream	I1	Dimethicone
		I2	None
		I3	Dimethicone
		I4	None
		I5	Dimethicone, Cyclopentasiloxane
		I6	None
		I7	None
		I8	Cyclopentasiloxane
		I9	Cyclopentasiloxane, Cyclohexasiloxane, Dimethicone
		N1	None
		N2	Cyclopentasiloxane, Dimethicone
	Facial cream	J1	Dimethicone
		J2	None
		J3	Cyclomethicone, Dimethicone

Table A3. Information present in the label of the products selected for the analysis of VMSs content (cont.).

Product Category	Product Subcategory	Code	cVMSs presence (as written in the ingredients list)
Moisturizers	Baby/children body lotion/milk/ cream	E1	None
		E2	Dimethicone
		E3	None
		E4	None
		E5	None
		E6	None
Deodorants/ antiperspirants	Roll–on deodorant	G1	None
		G2	None
		G3	None
		G4	None
		G5	None
		G6	None
		G7	None
		G8	None
		G9	None
		G10	None
		G11	None
		G12	None
Shaving products	Shaving foam/gel	K1	None
		K2	None
		K3	None
		K4	None
		K5	None
		K6	None
		K7	None
	Aftershave	L1	Cyclopentasiloxane, Dimethicone
		L2	None
		L3	Dimethicone
		L4	Dimethicone
Body and hair wash	Adult shower gel	F1	None
		F2	None
		F3	None
		F4	None
		F5	None
		F6	None
		F7	None
		F8	None
		F9	None
		M1	None
		M2	None
	Baby/children shower gel	H1	None
		H2	None
		H3	None
		H4	None
		H5	None
		H6	None
		H7	None
		H8	None
		H9	None

Table A3. Information present in the label of the products selected for the analysis of VMSs content (cont.).

Product Category	Product Subcategory	Code	cVMSs presence (as written in the ingredients list)
Body and hair wash	Adult shampoo	R1	None
		R2	None
		R3	Dimethicone
		R4	None
		R5	Dimethicone
		R6	Dimethicone
		R7	Dimethicone
		R8	None
		R9	Dimethicone
		R10	Dimethicone
		R11	None
		R12	Dimethicone
		R13	Dimethicone
		R14	Dimethicone
	Baby/children shampoo	R15	None
		S1	None
		S2	None
		S3	None
		S4	None
		S5	None
		S6	None
		S7	None
	Hair conditioner	T1	None
		T2	None
		T3	None
		T4	None
		T5	None
		T6	None
		T7	None
		T8	None
Hotel amenities	Shower gel	O1	None
		O2	None
		O3	None
	Shampoo	P1	None
		P2	None
		P3	None
		P4	None
	Body lotion/milk/cream	Q1	Dimethicone
		Q2	Cyclopentasiloxane, cyclotetrasiloxane
		Q3	None
		Q4	None
		Q5	None
		Q6	Dimethicone

Appendix 5. Daily dermal exposure

Table A4: Mean estimated daily dermal exposure in adults to siloxanes contained in selected toiletries.

					Mean								Mean										
Category	Product type	Amount per application ($g.event^{-1}$)	Frequency of application ($events.day^{-1}$)	Retention factor	Concentration of siloxanes ($\mu g.g^{-1}$)								Daily dermal exposure ($\mu g.kg_{bw}^{-1}.day^{-1}$)										
					L2	D3	L3	D4	L4	D5	L5	D6	L2	D3	L3	D4	L4	D5	L5	D6	Σ L2-L5	Σ D3-D6	Total
Moisturizers	Body lotion/milk/ cream	8.50	0.42	1.00	nd	4.06	<LOQ	22.98	0.23	203.06	0.29	117.47	0.00	0.24	0.00	1.37	0.01	12.08	0.02	6.99	0.03	20.68	20.71
	Hand cream	0.50	0.80	1.00	0.16	<LOQ	0.04	4.83	nd	3.30	0.47	0.87	0.00	0.00	0.00	0.03	0.00	0.02	0.00	0.01	0.01	0.06	0.06
	Facial cream	0.40	0.88	1.00	nd	0.47	<LOQ	13.82	<LOQ	250.07	nd	242.68	0.00	0.00	0.00	0.08	0.00	1.47	0.00	1.42	0.00	2.98	2.98
Toilet soaps	Solid soap	0.80	0.50	0.01	<LOQ	3.96	nd	0.71	nd	4.57	nd	5.27	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Gel soap	1.00	4.50	0.01	<LOQ	12.04	nd	1.17	nd	0.09	0.13	0.18	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
Body and hair wash	Shower gel	6.30	0.71	0.01	0.20	86.98	nd	25.67	nd	3.73	0.25	1.86	0.00	0.07	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.09	0.09
	Shampoo	4.80	0.50	0.01	0.28	341.25	0.25	91.45	0.84	18.36	0.80	17.98	0.00	0.14	0.00	0.04	0.00	0.01	0.00	0.01	0.001	0.19	0.19
	Hair conditioner	4.90	0.43	0.01	nd	19.60	0.15	34.03	0.64	19.35	0.54	28.46	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.001	0.04	0.04
Dentifrice products	Toothpaste	1.10	2.00	0.05	<LOQ	0.01	nd	0.02	nd	nd	nd	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Deodorants/ antiperspirants	Roll-on deodorant	0.20	1.00	1.00	nd	8.86	nd	2.87	<LOQ	1.34	nd	0.74	0.00	0.03	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.05	0.05
Shaving products	Shaving foam	3.55	0.43	0.01	nd	1.76	nd	<LOQ	nd	3.15	nd	2.12	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Aftershave	0.80	0.46	1.00	0.01	1.57	0.78	2.60	1.23	106.03	4.02	34.14	0.00	0.01	0.01	0.02	0.01	0.65	0.03	0.21	0.04	0.89	0.92
													TOTAL ($\mu g.kg_{bw}^{-1}.day^{-1}$)	0.00	0.50	0.01	1.58	0.02	14.24	0.05	8.65	0.08	24.97	**25.04**

Table A5: Maximum estimated daily dermal exposure in adults to siloxanes contained in selected toiletries.

					Maximum								Maximum									
Category	Product type	Amount per application (g.event^{-1})	Frequency of application (events.day^{-1})	Retention factor	Concentration of siloxanes (µg.g^{-1})								Daily dermal exposure (µg.kg$_{bw}^{-1}$. day^{-1})									
					L2	D3	L3	D4	L4	D5	L5	D6	L2	D3	L3	D4	L4	D5	L5	D6	Σ L2-L5	Σ D3-D6	Total
Moisturizers	Body lotion/milk/cream	8.50	0.42	1.00	nd	10.76	<LOQ	105.13	0.23	753.53	0.98	471.18	0.00	0.64	0.00	6.26	0.01	44.83	0.06	28.04	0.07	79.77	79.84
	Hand cream	0.50	0.80	1.00	0.16	<LOQ	0.04	12.37	nd	7.9	1.1	1.32	0.00	0.00	0.00	0.08	0.00	0.05	0.01	0.01	0.01	0.14	0.15
	Facial cream	0.40	0.88	1.00	nd	0.85	<LOQ	23.72	<LOQ	407.62	nd	594.24	0.00	0.01	0.00	0.14	0.00	2.39	0.00	3.49	0.00	6.02	6.02
Toilet soaps	Solid soap	0.80	0.50	0.01	<LOQ	4.39	nd	2.43	nd	9.16	nd	11.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Gel soap	1.00	4.50	0.01	<LOQ	22.01	nd	1.72	nd	0.27	0.13	0.3	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.02
Body and hair wash	Shower gel	6.30	0.71	0.01	0.2	309.59	nd	93.14	nd	15.74	0.25	6.5	0.00	0.23	0.00	0.07	0.00	0.01	0.00	0.01	0.00	0.32	0.32
	Shampoo	4.80	0.50	0.01	0.8	1203.28	0.39	267.03	1.34	39.94	1.28	42.01	0.00	0.48	0.00	0.11	0.00	0.02	0.00	0.02	0.00	0.62	0.62
	Hair conditioner	4.90	0.43	0.01	nd	40.42	0.25	117.36	0.98	49.46	0.78	62.31	0.00	0.01	0.00	0.04	0.00	0.02	0.00	0.02	0.00	0.10	0.10
Dentifrice products	Toothpaste	1.10	2.00	0.05	<LOQ	0.01	nd	0.05	nd	nd	nd	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Deodorants/ antiperspirants	Roll-on deodorant	0.20	1.00	1.00	nd	29.98	nd	10.72	<LOQ	3.58	nd	1.01	0.00	0.10	0.00	0.04	0.00	0.01	0.00	0.00	0.00	0.15	0.15
Shaving products	Shaving foam	3.55	0.43	0.01	nd	2.431	nd	<LOQ	nd	3.14	nd	3.96	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Aftershave	0.80	0.46	1.00	0.01	3.55	0.78	8.11	1.23	209.6	7.85	100.28	0.00	0.02	0.01	0.05	0.01	1.29	0.05	0.62	0.06	1.97	2.03
													TOTAL (µg.kg$_{bw}^{-1}$. day^{-1})	**0.00**	**1.51**	**0.01**	**6.78**	**0.02**	**48.62**	**0.12**	**32.19**	**0.14**	**89.11**	**89.25**

Table A6: Mean estimated daily dermal exposure in children to siloxanes contained in selected toiletries.

					Mean									Mean									
Category	Product type	Amount per application (g.event^{-1})	Frequency of application (events.day^{-1})	Retention factor	Concentration of siloxanes (µg.g^{-1})									Daily dermal exposure (µg.kg$_{bw}^{-1}$. day^{-1})									
					L2	D3	L3	D4	L4	D5	L5	D6		L2	D3	L3	D4	L4	D5	L5	D6	Σ L2-L5	Σ D3-D6	Total
Moisturizers	Body lotion/milk /cream	4.53	2.00	1.00	<LOQ	0.02	nd	0.07	nd	0.07	0.08	0.06		0.00	0.01	0.00	0.03	0.00	0.03	0.03	0.02	0.03	0.09	0.12
Toilet soaps	Solid soap	0.25	0.50	0.01	<LOQ	3.96	nd	0.71	nd	4.57	nd	5.27		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00
	Gel soap	0.29	4.50	0.01	<LOQ	12.04	nd	1.17	nd	0.09	0.13	0.18		0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
Body and hair wash	Shower gel	10.29	1.23	0.01	0.075	4.96	nd	2.81	0.13	2.44	0.45	4.43		0.00	0.03	0.00	0.02	0.00	0.01	0.00	0.03	0.01	0.09	0.09
	Shampoo	7.30	0.60	0.01	0.121	29.65	0.24	4.75	0.69	2.37	0.59	2.50		0.00	0.06	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.08	0.08
Dentifrice products	Toothpaste	0.53	2.00	1.00	<LOQ	0.31	nd	0.14	nd	0.13	nd	0.38		0.00	0.02	0.00	0.01	0.00	0.01	0.00	0.02	0.00	0.05	0.05
													TOTAL (µg.kg$_{bw}^{-1}$. day^{-1})	0.00	0.12	0.00	0.06	0.00	0.05	0.03	0.07	0.04	0.31	0.35

Table A7: Maximum estimated daily dermal exposure in children to siloxanes contained in selected toiletries.

					Maximum									Maximum									
Category	Product type	Amount per application (g.event^{-1})	Frequency of application (events.day^{-1})	Retention factor	Concentration of siloxanes (µg.g^{-1})									Daily dermal exposure (µg.kg$_{bw}^{-1}$. day^{-1})									
					L2	D3	L3	D4	L4	D5	L5	D6		L2	D3	L3	D4	L4	D5	L5	D6	Σ L2-L5	Σ D3-D6	Total
Moisturizers	Body lotion/milk /cream	4.53	2.00	1.00	<LOQ	0.02	nd	0.14	nd	0.15	0.08	0.08		0.00	0.01	0.00	0.06	0.00	0.06	0.03	0.03	0.03	0.16	0.19
Toilet soaps	Solid soap	0.25	0.50	0.01	<LOQ	4.39	nd	2.43	nd	9.16	nd	11.06		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Gel soap	0.29	4.50	0.01	<LOQ	22.01	nd	1.72	nd	0.27	0.13	0.30		0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02
Body and hair wash	Shower gel	10.29	1.23	0.01	0.11	8.01	nd	5.34	0.16	7.78	0.80	11.28		0.00	0.05	0.00	0.03	0.00	0.05	0.01	0.07	0.01	0.19	0.20
	Shampoo	7.30	0.60	0.01	0.18	43.79	0.41	20.13	1.00	7.39	0.87	4.41		0.00	0.09	0.00	0.04	0.00	0.01	0.00	0.01	0.01	0.15	0.16
Dentifrice products	Toothpaste	0.53	2.00	1.00	<LOQ	0.59	nd	0.30	nd	0.27	nd	0.56		0.00	0.03	0.00	0.02	0.00	0.01	0.00	0.03	0.00	0.08	0.08
													TOTAL (µg.kg$_{bw}^{-1}$. day^{-1})	**0.00**	**0.19**	**0.001**	**0.15**	**0.003**	**0.14**	**0.04**	**0.13**	**0.04**	**0.60**	**0.65**

Appendix 6: "Down-the-drain" emissions

Table A8: Estimated minimum *per capita* "down-the-drain" emissions of siloxanes.

Category	Product type	Estimated minimum *per capita* "down-the-drain" emissions of siloxanes (µg.day^{-1})							
		L2	D3	L3	D4	L4	D5	L5	D6
Moisturizers	Body lotion/milk/cream	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
	Hand cream	0.00	0.00	0.00	0.02	0.00	0.01	0.00	0.00
	Facial cream	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.01
Toilet soaps	Solid soap	0.00	0.00	0.00	0.04	0.00	0.04	0.00	0.00
	Gel soap	0.00	2.39	0.00	0.00	0.00	0.00	0.00	0.00
Body and hair wash	Shower gel	0.00	22.85	0.00	3.07	0.00	0.00	0.00	0.14
	Shampoo	0.00	14.12	0.00	4.36	0.00	0.76	0.00	0.79
	Hair conditioner	0.00	0.00	0.00	1.23	0.00	3.25	0.00	1.52
Dentifrice products	Toothpaste	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00
Deodorants/ antiperspirants	Roll-on deodorant	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Shaving products	Shaving foam/gel	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Aftershave	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Total (µg.day^{-1})	**0.00**	**39.36**	**0.00**	**8.74**	**0.00**	**4.12**	**0.00**	**2.48**

Table A9: Estimated average *per capita* "down-the-drain" emissions of siloxanes.

Category	Product type	Estimated average per capita "down-the-drain" emissions of siloxanes (µg.day^{-1})							
		L2	D3	L3	D4	L4	D5	L5	D6
Moisturizers	Body lotion/milk/cream	0.00	0.65	0.00	3.69	0.04	32.62	0.05	18.87
	Hand cream	0.00	0.00	0.00	0.09	0.00	0.06	0.01	0.01
	Facial cream	0.00	0.01	0.00	0.22	0.00	3.96	0.00	3.84
Toilet soaps	Solid soap	0.00	1.57	0.00	0.28	0.00	1.81	0.00	2.09
	Gel soap	0.00	53.65	0.00	5.22	0.00	0.41	0.59	0.81
Body and hair wash	Shower gel	0.87	385.33	0.00	113.72	0.00	16.51	1.10	8.26
	Shampoo	0.66	811.17	0.60	217.38	1.99	43.64	1.90	42.74
	Hair conditioner	0.00	40.89	0.32	71.02	1.34	40.39	1.13	59.39
Dentifrice products	Toothpaste	0.00	0.02	0.00	0.05	0.00	0.00	0.00	0.08
Deodorants/ antiperspirants	Roll-on deodorant	0.00	0.08	0.00	0.03	0.00	0.01	0.00	0.01
Shaving products	Shaving foam/gel	0.00	2.66	0.00	0.00	0.00	4.76	0.00	3.21
	Aftershave	0.00	0.03	0.01	0.04	0.02	1.76	0.07	0.57
	Total (µg.day^{-1})	**1.53**	**1,296.05**	**0.93**	**411.73**	**3.39**	**145.93**	**4.85**	**139.87**

Table A10: Estimated maximum *per capita* "down-the-drain" emissions of siloxanes.

Category	Product type	Estimated maximum *per capita* "down-the-drain" emissions of siloxanes (µg.day^{-1})							
		L2	D3	L3	D4	L4	D5	L5	D6
Moisturizers	Body lotion/milk/cream	0.00	1.73	0.00	16.89	0.04	121.05	0.16	75.70
	Hand cream	0.00	0.00	0.00	0.22	0.00	0.14	0.02	0.02
	Facial cream	0.00	0.01	0.00	0.38	0.00	6.46	0.00	9.41
Toilet soaps	Solid soap	0.00	1.74	0.00	0.96	0.00	3.63	0.00	4.38
	Gel soap	0.00	98.08	0.00	7.68	0.00	1.18	0.59	1.35
Body and hair wash	Shower gel	0.00	47.68	0.00	465.77	1.04	3338.34	4.32	2087.46
	Shampoo	1.91	2860.29	0.92	634.75	3.19	94.94	3.03	99.86
	Hair conditioner	0.00	84.35	0.51	244.92	2.04	103.22	1.63	130.03
Dentifrice products	Toothpaste	0.00	0.03	0.00	0.10	0.00	0.00	0.00	0.19
Deodorants/ antiperspirants	Roll-on deodorant	0.00	0.27	0.00	0.10	0.00	0.03	0.00	0.01
Shaving products	Shaving foam/gel	0.00	3.68	0.00	0.00	0.00	4.76	0.00	5.99
	Aftershave	0.00	0.06	0.01	0.13	0.02	3.47	0.13	1.66
	Total (µg.day^{-1})	**1.91**	**3,097.91**	**1.45**	**1,371.89**	**6.32**	**3,677.22**	**9.89**	**2,416.06**

Appendix 7: Presentations and publications in the scope of this project

Encontro de Jovens Investigadores da Universidade do Porto - 8ª edição, 13-15 May 2015, Porto (Portugal) - Oral presentation

New analytical methodology based on QuEChERs followed by GC-MS to determine siloxanes in personal care products

D. Capela, L. Santos and V. Homem
LEPABE, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Portugal.

Siloxanes are organic compounds used in a wide variety of personal care products, such as shampoos, hair conditioners, lotions, etc. Chemically, they consist of a structural unit of alternating Si-O bond linked into a cyclic or linear way, with organic side chains [1]. They are usually incorporated in toiletries as antifoaming and conditioning agents, film formers and emollients. These compounds have sparked a growing interest in the scientific community since some laboratory studies in animals have shown that siloxanes may have toxic effects [1]. In fact, these compounds have already been detected in biological matrices such as human blood and breast milk [2]. Due to their ubiquitous occurrence, bioaccumulation and toxic potential, siloxanes are compounds of concern for human health, mainly through dermal exposure by toiletries application. Although several studies have been conducted to determine siloxanes in environmental and human compartments, few have focused on their determination in personal care products. Therefore, the aim of this study was to develop and validate a methodology based on quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction followed by GC–MS for the analysis of 8 siloxanes in different toiletries.

The selected methodology uses hexane as extraction solvent and two QuEChERS, one containing $MgSO_4$ and $NaCH_3COO$ to promote phase separation and other containing a mixture of $MgSO_4$, PSA bonded silica and C18 to remove undesired components. Chromatographic analyses were performed by a Varian Ion Trap GC-MS system.

Personal care products were divided into different categories according to their overall composition (moisturizers, hair care products, body wash, shaving products, dentifrices, deodorants, perfumes and toilet soaps). Low detection limits, high average recoveries (>80%) and precision (RSD <15%) were determined. In general, higher levels of siloxanes were detected in shampoos and hair conditioners. Cyclic siloxanes were detected more frequently in the analyzed products.

This work was funded by FEDER funds through the Operational Programme for Competitiveness Factors – COMPETE, ON.2 - O Novo Norte - North Portugal Regional Operational Programme and National Funds through FCT - Foundation for Science and Technology under the projects: PEst-C/EQB/UI0511, NORTE-07-0124-FEDER-000025 - RL2_ Environment & Health. Vera Homem would also like to thank FCT for the post-doctoral grant SFRH/BPD/76974/2011 co-funded by QREN-POPH.

References:

[1] Lu, Y., Yuan, T., Wang, W. and Kannan, K. (2011), *Concentrations and assessment of exposure to siloxanes and synthetic musks in personal care products from China*, Environmental Pollution, 159 (12), 3522-3528.

[2] Zhang, X., Jing, Y., Zhou, J., Fang, X., Zhang, X. and Yu, Y. (2015), *Occurrence and transport of synthetic musks in paired maternal blood, umbilical cord blood, and breast milk*, International Journal of Hygiene and Environmental Health, 218 (1), 99-106.

Short-seminar "Siloxanes in cosmetics and personal care products", FEUP, Product Chemistry and Technology, Master in Bioengineering and Chemical Engineering, 13 October 2015

Siloxanes in cosmetics and personal care products

Index

- Siloxanes – An overview
  - Definition
  - Applications
  - Physicochemical properties
  - Legislation
  - Toxicity
- Analytical methodology
  - QuEChERS (extraction and clean-up method)
  - Instrumental method (GC-MS)
- Conclusions

October 13th 2015

Daniela Capela
dcapela@fe.up.pt

European Meeting on Environmental Chemistry (EMEC 16), 30 November-3 December 2015, Torino (Italy) - Oral presentation

AN - 13

Assessment of siloxanes release into the environment by personal care products

[1]Vera Homem, [1]Daniela Capela, [1]Arminda Alves, [1]Lúcia Santos

[1]LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy - Faculty of Engineering, University of Porto, Porto (Portugal)

vhomem@fe.up.pt

Siloxanes are organic compounds that contain a backbone of alternating Si-O units linked in a cyclic or linear way, with methyl side chains attached to each silicon atom [1]. Due to their low surface tension, high thermal stability and smooth texture, they have been used in a wide variety of personal care products (PCPs), such as shampoos, hair conditioners, lotions, etc. Recent studies show that siloxanes may have toxic effects on aquatic organisms, but also on mammals. In fact, these compounds are suspected of causing impaired fertility, liver damage and oestrogen mimicry [2]. Due to their ubiquitous occurrence, bioaccumulation and toxic potential, siloxanes are compounds of concern for both environmental and human health. Therefore, it is essential to examine their concentrations in PCPs to enable a prediction of human and environmental exposure by measuring their inputs.

In this work, an analytical method based on quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction followed by GC–MS was used for the analysis of eight siloxanes in PCPs. These products were chosen according to the usage patterns of the Oporto population (Portugal). Low detection limits, high average recoveries (>80%) and precision (RSD <15%) were determined.

Siloxanes were detected in most analysed samples in a wide range of concentrations. In general, higher levels of siloxanes were detected in shampoos and hair conditioners. Cyclic siloxanes were determined more frequently in the analyzed products. The human daily exposure to siloxanes was estimated, as well as the release rates of these compounds into the environment. Wash-off products represent the greater emission source.

Acknowledgments. This work was financially supported by: Project UID/EQU/00511/2013-LEPABE, by the FCT/MEC with national funds and when applicable co-funded by FEDER in the scope of the P2020 Partnership Agreement; Project NORTE-07-0124-FEDER-000025 - RL2_ Environment&Health, by FEDER funds through Programa Operacional Factores de Competitividade – COMPETE, by the Programa Operacional do Norte (ON2) and by national funds through FCT - Fundação para a Ciência e a Tecnologia (Scholarship SFRH/BPD/76974/2011).

References:

[1] Hong, W.-J., Jia, H., Liu, C., Zhang, Z., Sun, Y., Li, Y.-F., Environ. Pollut. 191 (2014) 175-181.

[2] Wang, D.-G., Norwood, W., Alaee, M., Byer, J.D., Brimble, S., Chemosphere 93 (2013) 711–725.

XVI COLACRO/9º Encontro Nacional de Cromatografia, 5-9 January 2016, Lisbon (Portugal) - Oral presentation

QUECHERS OF MICROPOLLUTANTS: MISSION IN SEVERAL MATRICES

N. Ratola[a],*, V. Homem[a], D. Capela[a], S. Ramos[a], J.A. Silva[a], C. Cunha[a], E. Silva[a], I. Magalhães[a], R. Araújo[a], L. Santos[a] and A. Alves[a]

[a] LEPABE, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, Porto, Portugal

QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) is a recent kind of dispersive solid-phase extraction (d-SPE) that offers several advantages over traditional methods: (a) fast and easy to perform and therefore less susceptible to error; (b) uses disposable containers and devices, minimising the risk of contamination; (c) low consumption of resources (solvents, sorbents, working time) and easy handling. A typical QuEChERS protocol involves extraction, partitioning and cleanup steps [1]. The most commonly employed sorbents are primary secondary amine (PSA), octadecylsilica (C18) and graphitized carbon black [2]. The selection of the appropriate sorbents and their proportions is crucial to achieve best analytical performance and there is a wide choice of pre-prepared QuEChERS mixtures commercially available, in polypropylene tubes or sachets. However, it was proven that QuEChERS prepared in the lab can show similar performances to the commercial ones [3]. In this work we show their applicability in the assessment of the levels of several semi-volatile organic micro-pollutants (PAHs, PCBs, PBDEs, pesticides, musks and siloxanes) found in some matrices usually difficult to handle: vegetation, soils, personal care products (PCPs) and sand. The analysis were performed by our work group under different projects, but rely on a common approach: two QuEChERS were prepared and used sequentially after a previous sonication step and before quantification by GC/MS: QuEChERS 1 (Q1) was used in the partitioning step and contained $MgSO_4$ and of CH_3COONa. QuEChERS 2 (Q2) was used for the cleanup of the extract and contained $MgSO_4$, PSA and C18. This technique represented a step forward in terms of environmentally-friendly laboratory policies and time consumption, with no observable loss of performance in

comparison with the classic extraction methods. An overview of the mean recoveries obtained for each family of compounds and each matrix is presented in Table 1.

Table 1. Mean recoveries (%) of analytical approaches using QuEChERS

	PAHs	PCBs	PBDEs	Pesticides	Musks	Siloxanes
Pine needles	83	89	86	91	79	75
Soil	88	94	99	87	87	69
PCPs	-	-	-	-	85	87
Sand	-	-	-	-	95	-

Acknowledgements: This work was financially supported by: Project UID/EQU/00511/2013-LEPABE (Laboratory for Process Engineering, Environment, Biotechnology and Energy – EQU/00511) by FEDER funds through Programa Operacional Factores de Competitividade e Internacionalização – COMPETE2020 and by national funds through FCT - Fundação para a Ciência e a Tecnologia; Project Reference EXPL/AAG-MAA/0981/2013, Investigador FCT contract IF/01101/2014 (Nuno Ratola) and post-doctoral grant SFRH/BPD/76974/2011 (Vera Homem). Many thanks to all the institutions and volunteers that helped in the choice/maintenance of the sampling sites.

References:

[1] O.D. Prestes, M.B. Adaime, R. Zanella. Sci. Chromatogr. 2011, 3, 51-64.

[2] UCT - United Chemical Technologies, Inc. QuEChERS Informational Booklet, Bristol, PA, USA. 2014, 24 p. Available at https://www.unitedchem.com/sites/default/files/docs/product-displays/QuEChERS_Booklet_2014.pdf.

[3] V. Homem, J.A. Silva, C. Cunha, A. Alves, L. Santos. J. Sep. Sci. 2013, 36, 2176-2184.

Paper submitted to the peer-reviewed journal "Environment International", Ms. Ref. No.: ENVINT-D-15-01732

From the shop to the drain - Volatile methylsiloxanes in cosmetics and personal care products

Daniela Capela, Arminda Alves, Vera Homem, Lúcia Santos

LEPABE — Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal

*Corresponding author. Tel.: +351 22 041 4947, Fax: +351 22 508 1449, e-mail address: vhomem@fe.up.pt

Abstract

Organosiloxanes are extremely used in the formulation of a wide range of cosmetic and personal care products, including creams and lotions, bath soaps, shampoo and hair care products to soften, smooth, and moisten. In fact, the intensive and widespread use of organosiloxanes combined with their lipophilic nature, makes them interesting targets for future research, particularly in the toxicology area.

This study focused on determining the concentration levels of these compounds in the bestselling brands of personal care products (PCPs) in the Oporto region (Portugal), allowing the estimation of dermal exposure to siloxanes and the evaluation of the quantities released to the environment "down-the-drain". To accomplish this task, a QuEChERS technique ("Quick, Easy, Cheap, Effective, Rugged, and Safe") was employed to extract the siloxanes from the target personal care products, which was never tested before. The resulting extract was analysed by gas chromatography-mass spectrometry (GC-MS). The limits of detection varied between 0.17 (L2) and 3.75 ng g^{-1} (L5), being much lower than any values reported in the literature for this kind of products. In general, satisfactory precision (<10%) and accuracy values (average recovery of 84%) were obtained.

123 PCPs were analysed (moisturizers, deodorants, body and hair washes, toilet soaps, toothpastes and shaving products) and volatile methylsiloxanes were detected in 96% of the samples, in concentrations between 0.003 µg g^{-1} and 1,203.28 µg g^{-1}. Shampoo exhibited the highest concentration for cyclic and aftershaves for linear siloxanes. Combining these results with the daily usage amounts, an average daily dermal exposure of 25.04 µg kg_{bw}^{-1} day^{-1} for adults and 0.35 µg kg_{bw}^{-1} day^{-1} for baby/children was achieved. The main contributors for adult dermal exposure were body moisturizers, followed by facial creams and aftershaves, while for baby/children were body moisturizers, followed by shower gel and shampoo. An estimate of the amount of siloxanes released "down-the-drain" into the sewage systems through the use of toiletries was also performed. An emission *per capita* between 54.71 and 10,606.93 µg day^{-1} (mean: 2,011.05 µg day^{-1}) is expected and shampoo and shower gel presented the higher mean total values (1,120.13 µg day^{-1} and 525.82 µg day^{-1}, respectively). In the worst-case scenario, D5 and D3 were the predominant siloxanes in the effluents with 3,690.50 µg day^{-1} and 3,098.25 µg day^{-1}, respectively.

Keywords: volatile methylsiloxanes, personal care products, human dermal exposure, down-the-drain emissions, QuEChERS, GC-MS